

THE ROLE OF INTERTIDAL HEIGHT, MICROHABITAT USE AND MOTILITY ON
THE ONTOGENY OF STRESS TOLERANCE TO AIR TEMPERATURE AND
DESICCATION IN SIX INTERTIDAL INVERTEBRATE SPECIES

by

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ABSTRACT

High temperature and desiccation are particularly significant sources of mortality for intertidal invertebrates which are exposed to terrestrial conditions daily at low tide. Motility can play a role in tolerance of environmental stress, especially in juveniles which are more susceptible than adults to many mortality factors. Certain lifestyle characteristics and physiological traits change throughout life in response to the changing requirements of an animal throughout its life cycle. This study thus investigates the role of intertidal range, microhabitat and motility on stress tolerance by determining how tolerance thresholds to temperature and desiccation differ between newly metamorphosed juveniles and individuals of full adult size for six intertidal invertebrates.

To determine a species' tolerance to temperature and identify potential ontogenetic shifts in tolerance thresholds, juveniles and adults of four species of intertidal invertebrates (*Balanus glandula*, *Chthamalus dalli*, *Nucella ostrina* and *Mytilus trossulus*) were subjected to temperature treatments in the laboratory. In each of these four species, the temperature tolerance threshold (LT₅₀) increased by 0.2°C (*C. dalli*) – 3.3°C (*M. trossulus*) from early juvenile to adult, indicating a modest ontogenetic shift in temperature tolerance. In desiccation tolerance experiments the previous four species were tested along with *Littorina scutulata* and *Petrolisthes cinctipes*. All species demonstrated an increase in desiccation tolerance threshold throughout their ontogeny, ranging from an increase of 183 (*C. dalli*) - 26,100 % (*M. trossulus*).

The magnitude of the ontogenetic shifts in temperature and desiccation tolerance thresholds differed among species, as did tolerance thresholds but these differences were not related to a sessile or motile life strategy except in juvenile temperature tolerance as identified with a Pearson's product-moment correlation analysis. Desiccation tolerance thresholds of juveniles were more closely related to the upper limit of intertidal range and microhabitat use. Adult temperature and desiccation tolerance were related to the upper limit of the intertidal range and microhabitat. While motility may not be a defining factor of the ontogeny of intertidal invertebrates, these findings do suggest that juveniles of all species tested are more susceptible to temperature and desiccation stress than adults, and thus indicates that certain niche dimensions, particularly intertidal range and microhabitat, are

associated with an animal's ability to tolerate stress. Although there are no obvious patterns linking stress tolerance and motility, species show very different degrees of ontogenetic shift in tolerance. The extensive interspecific variation in the magnitude of shifts is likely a complex outcome of differences in intertidal range, microhabitat shift and tolerance to specific stressors not yet investigated.

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CHAPTER 1: General Introduction

Tolerance to environmental stress is critical for invertebrates living in the rocky intertidal ecosystem. Invertebrates living along wave-exposed shores experience a range of biotic and abiotic stressors and subsequently exhibit a broad diversity of behaviours and life histories adapted to survive these harsh conditions. When the tolerance thresholds to these stressors are exceeded by environmental conditions, mortality occurs, which can result in changes in population abundance, species composition and habitat structure, ultimately altering community diversity (Seapy and Littler, 1982). The causes of population fluctuations have been attributed to duration of larval period (Eckert, 2008) and larval supply (Yoshioka, 1982) though processes occurring beyond the larval stage can also impact populations. Following settlement and metamorphosis, a number of environmental stressors impact population distribution, abundance and community structure when mortality occurs (McQuaid, 1982; Rowley, 1989; Sorte et al., 2011). The role and the importance of each of these mortality factors, however, are less understood.

Behaviour, morphology and physiology are outcomes, in part, of the evolutionary pressures that stressors impart, allowing intertidal animals to tolerate a range of challenging conditions. Particularly characteristic of an intertidal ecosystem are broad changes in temperature, high levels of solar radiation, desiccation, algal whiplash, and predation pressure (Gosselin and Chia, 1995; Gosselin and Jones, 2010; Gosselin and Qian, 1997; Jenewein and Gosselin, 2013a). These fluctuations can occur daily, monthly and yearly; as a result, shifts in behaviour, population abundance and community structure can vary on similar timescales. Species whose vertical range of distribution lies within the intertidal zone are unique in that, despite being of marine origin, they must effectively survive and tolerate terrestrial conditions for periods exceeding 6 h, twice daily. These terrestrial conditions may result in biotic stressors such as predation from land mammals and birds, but also result from the effects of abiotic stressors such as high air temperature or desiccation that are important sources of mortality for intertidal invertebrates (Denny et al. 2006, Fenberg et al. 2015, Jenewein & Gosselin 2013, Mislán et al. 2014).

With drastic changes in temperature and water availability occurring every low tide period, if animals cannot find shelter or adjust their behaviour or physiology sufficiently to

tolerate the stress, mortality may occur. Temperature changes can affect chemical processes within the body, the viscosity of body fluids (e.g. blood, mucus), growth, and the solubility of oxygen (Moore, 1958). Desiccation, a result of several environmental conditions including wind, heat and humidity, is also of extreme importance to the survival of intertidal invertebrates because water is essential for gas exchange (Foster, 1971). With water evaporation, salinity can also increase (Newell, 1976) causing hyperosmotic stress to cellular processes in an organism.

Although high temperature or desiccation may not always result in mortality, when conditions exceed the tolerance limits of an organism, higher tolerance is selected and evolutionary changes in life processes occur. The resulting modifications require a shift in energy allocation and therefore could indirectly cause mortality due to increased stress (Vermeij, 1987). Given that temperature and desiccation are major sources of mortality, they likely play an important role in the evolution of intertidal invertebrates, potentially changing the ontogeny, ecology or morphology of a species. While several previous studies have identified environmental stressors and evolutionary pressures in the intertidal ecosystem, few studies have investigated how those pressures affect different species and how the importance of these factors change throughout ontogeny.

While mortality plays a role in shaping population dynamics and community structure, the timing of this mortality in the animal's life cycle is particularly significant to the evolution of the species' life history. In larval stages, mortality is high and variation in mortality between groups or populations at this stage can affect the distribution of species, genetic exchange among populations, recruitment, and population abundance (Cowen and Sponaugle, 2009; Marshall and Keough, 2004; Pineda et al., 2009). Other work has shown that the period of life immediately following metamorphosis, from a larval stage or hatching from an egg capsule, is also a period of high mortality. Juveniles are particularly vulnerable in many intertidal invertebrate species, with the most critical period for survival occurring in the first 24 h post-metamorphosis or hatching (Gosselin and Qian, 1996, 1997; Guillou and Tartu, 1994; Hunt and Scheibling, 1997); this period of life is referred to as the early benthic phase. The high mortality that occurs during this early benthic phase suggests it is ecologically distinct from planktonic and adult stages (Gosselin and Qian, 1997) and

indicates the early benthic phase may be critical in determining fluctuations in population abundance.

The factors causing early benthic phase mortality are of high ecological and evolutionary importance. Populations are regulated by processes that determine the input of new individuals and the removal of potential recruits from an existing population (Hughes, 1990). Mortality in early life stages can have a significant role in regulating population size (Connell, 1985) as it directly alters the number of new individuals entering the population. For example, Connell (1985) showed that post-settlement mortality in barnacles directly impacted the size of their populations. While the number of potential settlers entering a population is important to population abundance and density, if these settlers encounter stress that exceeds tolerance limits during the early benthic phase they will not reach reproductive maturity (Fraschetti et al., 2002). Furthermore, if early mortality is unequal among species in a certain geographical area, then community structure can also be affected along with the population abundance of a given species. Since high temperature and desiccation are important sources of mortality in the intertidal zone, these factors likely influence population dynamics and community structure as well as the evolution and life history of these intertidal invertebrates.

The early benthic phase may be critical for survival in some species since vulnerability is high during this life stage. Additionally, the importance of intertidal temperature or desiccation on this early benthic period could vary depending on the life strategy of a species. Despite the unique niche that intertidal invertebrates have colonised, between land and sea, these animals are immensely diverse and represent as many as 10 phyla. Within the intertidal zone, animals display numerous morphological forms, reproductive modes, feeding strategies, and differ in habitat selection, behaviour and size. With this variation comes a range of stress tolerance mechanisms that allow these animals to occupy specific niches within the intertidal habitat. Of obvious note is the difference in motility levels of intertidal invertebrates. In addition, some, such as barnacles, mussels and anemones, remain either entirely sessile or sedentary throughout post-larval life. Others such as snails, crabs, worms and limpets, are fully motile throughout life. With the ability to move, there is no need for motile animals to rely solely on physiological tolerance to stressors for

survival, whereas sessile animals cannot so easily avoid stress and must rely more heavily on physiological mechanisms and form.

Along with other lifestyle characteristics, the ability of an animal to move may change throughout life. One example of this is the mussel, *Mytilus trossulus*, which is motile as a juvenile but becomes sedentary as an adult (Bayne, 1964). In all animals, ontogenetic shifts are common and are demonstrated in changes in biology, ecology and physiology. These changes often occur in concert with one another and represent a change in environmental pressures important to an animal not only throughout life, but also among individuals. By studying physiological tolerance throughout life, other factors in an animal's lifestyle that may be correlated to changes in stress tolerance can be assessed. The primary goal of this research is to determine whether high temperature and desiccation play a significant role in the ecology of representative intertidal species by exerting a pressure significant enough to result in an ontogenetic shift in tolerance. A secondary goal is to determine if the relative importance of these stressors differs between motile and sessile species.

Chapter 2 will investigate interspecific and ontogenetic variation in tolerance thresholds of representative intertidal invertebrates to high temperature and desiccation through a series of controlled experiments. By studying a species throughout its life, it can be determined whether juvenile vulnerability to these stressors is apparent and whether an ontogenetic shift occurs in tolerance limits. In that chapter I will also investigate the relevance of motility, intertidal zonation, and microhabitat use for temperature and desiccation tolerance, to better understand the evolutionary role of life strategies and characteristics. Finally, Chapter 3 will provide a summary of the findings and investigate the implications of these findings for populations of invertebrates in the Pacific Northwest rocky intertidal zone.

LITERATURE CITED

- Bayne BL (1964) Primary and Secondary Settlement in *Mytilus edulis* L. (Mollusca). *J Anim Ecol* 33:513–523
- Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. *J Exp Mar Bio Ecol* 93:11–45
- Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Ann Rev Mar Sci* 1:443–466
- Denny MW, Miller LP, Harley CDG (2006) Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *J Exp Biol* 209:2420–2431
- Eckert GL (2008) Effects of the Planktonic Period on Marine Population Fluctuations. *Ecol Soc Am* 84:372–383
- Fenberg PB, Menge BA, Raimondi PT, Rivadeneira MM (2015) Biogeographic structure of the northeastern Pacific rocky intertidal: The role of upwelling and dispersal to drive patterns. *Ecography (Cop)*:83–95
- Foster BA (1971) Desiccation as a factor in the intertidal zonation of barnacles. *Mar Biol* 8:12–29
- Fraschetti S, Giangrande A, Terlizzi A, Boero F (2002) Pre- and post-settlement events in benthic community dynamics. *Oceanol Acta* 25:285–295
- Gosselin LA, Chia FS (1995) Characterizing temperate rocky shores from the perspective of an early juvenile snail: the main threats to survival of newly hatched *Nucella emarginata*. *Mar Biol* 122:625–635
- Gosselin LA, Jones LA (2010) Effects of solar radiation on barnacle settlement, early post-settlement mortality and community development in the intertidal zone. *Mar Ecol Prog Ser* 407:149–158
- Gosselin LA, Qian PY (1996) Early post-settlement mortality of an intertidal barnacle: A critical period for survival. *Mar Ecol Prog Ser* 135:69–75

- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- Guillou J, Tartu C (1994) Post-larval and juvenile mortality in a population of the edible cockle *Cerastoderma edule* (L.) from northern Brittany. *Netherlands J Sea Res* 33:103–111
- Hughes TP (1990) Recruitment limitation, mortality, and population regulation in open systems: A case study. *Ecology* 71:12–20
- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar Ecol Prog Ser* 155:269–301
- Jenewein BT, Gosselin LA (2013) Early benthic phase mortality of the barnacle *Balanus glandula* is influenced by *Fucus* spp. cover but not by weather-related abiotic conditions. *J Exp Mar Bio Ecol* 449:28–35
- Marshall DJ, Keough MJ (2004) Variable effects of larval size on post-metamorphic performance in the field. *Mar Ecol Prog Ser* 279:73–80
- McQuaid CD (1982) The influence of desiccation and predation on vertical size gradients in populations of the gastropod *Oxysteles variegata* (Anton) on an exposed rocky shore. *Oecologia* 53:123–127
- Mislan KAS, Helmuth B, Wethey DS (2014) Geographical variation in climatic sensitivity of intertidal mussel zonation. *Glob Ecol Biogeogr* 23:744–756
- Moore HB (1958) *Marine ecology*. John Wiley & Sons, New York
- Newell RC (1976) Adaptation to Environment. In: Newell RC (ed) *Adaptation to Environment: Essays on the Physiology of Marine Animals*. Butterworth & Co (Publishers) Ltd., p 1–82
- Pineda J, Reynolds NB, Starczak VR (2009) Complexity and simplification in understanding recruitment in benthic populations. *Popul Ecol* 51:17–32

Rowley RJ (1989) Settlement and recruitment of sea urchins (*Strongylocentrotus* spp.) in a sea-urchin barren ground and a kelp bed: are populations regulated by settlement or post-settlement processes? *Mar Biol* 100:485–494

Seapy RR, Littler MM (1982) Population and Species Diversity Fluctuations in a Rocky Intertidal Community Relative to Severe Aerial Exposure and Sediment Burial. *Mar Biol* 71:87–96

Sorte CJB, Jones SJ, Miller LP (2011) Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *J Exp Mar Bio Ecol* 400:209–217

Vermeij GJ (1987) *Evolution and Escalation: An Ecological History of Life*. Princeton University Press

Yoshioka PM (1982) Role of Planktonic and Benthic Factors in the Population Dynamics of the Bryozoan *Membranipora Membranacea*. *Ecology* 63:457–468

CHAPTER 2: Role of motility, microhabitat use and intertidal height on ontogeny of temperature and desiccation tolerance in intertidal invertebrates.

INTRODUCTION

Benthic invertebrates living in the rocky intertidal ecosystem, at the interface of marine and terrestrial ecosystems, experience a number of unique challenges to survival associated with aerial exposure at low tide, during which they are exposed to terrestrial conditions for periods that can exceed 8 h, twice daily. Of particular concern for these animals is the physiological stress encountered at low tide, with desiccation and temperature generally being recognized as the most significant sources of physiological stress (Bertness et al., 1999; Gosselin and Chia, 1995; Miller et al., 2009; reviewed by Tomanek and Helmuth, 2002). Young individuals are especially vulnerable to temperature and desiccation, which contribute to high mortality in the early benthic phase (Gosselin and Qian, 1997; Jenewein and Gosselin, 2013a, 2013b) but this mortality decreases considerably in late juvenile and adult phases (Gosselin and Qian, 1997). This ontogenetic decrease in mortality suggests a possible corresponding ontogenetic shift in physiological tolerance to temperature and desiccation stress. The intertidal zone is also a highly heterogeneous habitat, such that nearby individuals can experience different environmental conditions depending on their intertidal zonation, microhabitat use, and motility (Tomanek and Helmuth, 2002). This brings into question whether stress tolerance varies accordingly among species. To our knowledge, the extent of interspecific variation in stress tolerance among sympatric intertidal species, as well as variation in the extent of ontogenetic shifts in stress tolerance, have not been investigated. In addition, the role of vertical distribution in the intertidal zone, microhabitat use, and motility in determining stress tolerance is currently unknown.

Benthic invertebrates have physiological, morphological and behavioural mechanisms for stress tolerance that allow them to withstand high temperatures and desiccation associated with low tide periods, particularly the extreme conditions experienced in summer months. Physiological responses can include a slowing of metabolism, upregulation in production of protective proteins, or adaptive shifts in systems reliant on energy budgets so that animals can allocate more energy to physiological tolerance against sources of stress (Hayward et al., 2004; Somero, 2002). The mechanisms by which intertidal animals achieve higher tolerance

to elevated temperature and desiccation, involving physiological responses as well as the growth of protective body structures and the growth to a larger body size, are energetically costly. For example, when experiencing high temperatures and desiccation, oxygen uptake was reduced by as much as 87% in mussels (Marsden and Weatherhead, 1998) and the solubility of oxygen decreased; these changes present additional stress to aerobic metabolism (Somero, 2002), slowing ATP production (Sokolova, 2013). Replacing denatured proteins damaged through high temperatures (reviewed by Sokolova, 2013; snails, Somero, 2002) as well as the activation and upregulation of heat shock protein production under high temperatures and desiccation (Hayward et al., 2004) also incur a high energetic cost. A tradeoff therefore exists between the benefits of stress tolerance mechanisms, particularly improved survival and growth, and the energetic costs of these mechanisms. Consequently, one could expect that intertidal animals would evolve mechanisms of stress tolerance that are proportional to the unique conditions they would encounter in their environment.

One effective mechanism for desiccation tolerance can be rapid growth to reach large sizes. Desiccation tolerance generally increases with body size because a large body size contains more water and also because the surface area to volume ratio decreases with increasing body size (Lowell, 1984). Temperature tolerance, on the other hand, might not be related to body size, as observed by Wolcott (1973) for several limpet species. However, the size and shape of animals varies among species and thus the impact of size on stress tolerance likely varies among morphologically different groups.

Although different species use similar mechanisms for stress tolerance, such as evaporative cooling or reduced metabolic rate, closely-related animals may nevertheless inhabit different niches, for example occupying different vertical ranges within the intertidal zone, which would expose these animals to different levels of stress. Evidence to date suggests animals living high in the intertidal zone are more tolerant of extreme temperatures and desiccation than individuals occurring lower in the intertidal zone (Miller et al., 2009; Mislán et al., 2014; Stillman and Somero, 1996); however, few studies have investigated the relationship between intertidal range and interspecific variation in tolerance. Variation in stress tolerance may also be associated with microhabitat use; in a study of 10 species living in rocky intertidal habitats of the North Atlantic, Kensler (1967) found that species living in microhabitats exposed to open air were more tolerant of high air temperature and desiccation

than those living in cryptic habitats. Additionally, motility allows some intertidal invertebrates to control their exposure to stressful conditions; some, such as crabs and snails, are highly motile, enabling them to escape the most stressful conditions by taking refuge, whereas others, such as barnacles, are completely sessile from the onset of benthic life, and thus are unable to escape extreme temperature or desiccation conditions. For example *Balanus glandula* and *Chthamalus dalli*, two barnacle species, are sessile from the onset of benthic life and cannot move to less stressful locations, whereas the snail *Nucella ostrina* or the crab *Petrolisthes cinctipes* are fully motile and able to choose foraging times to minimize exposure to high temperature or desiccation during low tide. Accordingly, some sessile species experience higher levels of early benthic phase mortality than motile species (Hunt and Scheibling, 1997). Thus, sessile species might be expected to have evolved more effective physiological and anatomical defenses against extreme temperature and desiccation.

Some species, such as *Mytilus trossulus*, undergo an ontogenetic shift in motility, beginning benthic life as a fully motile form moving through a cryptic habitat, then undergoing a secondary dispersal, and finally becoming sessile for the remainder of life (Bayne, 1964; Martel et al., 2000). Thus, in some cases, changes in motility might result in a change in exposure to high temperatures and desiccation and thus an ontogenetic shift in physiological tolerance would be required for survival. The above examples of variation in temperature and desiccation experienced by intertidal invertebrates resulting from three different niche dimensions (intertidal height, microhabitat and motility) suggest interspecific variation in tolerance thresholds may be linked to differences in habitat selection and life strategy. In addition, for some species, the use of different niches varies throughout life and thus may correlate to ontogenetic shifts in tolerance thresholds.

Desiccation and high temperatures can be a significant source of mortality in intertidal invertebrates immediately following metamorphosis during the early benthic phase (Gosselin and Qian, 1997; Hunt and Scheibling, 1997; Jenewein and Gosselin, 2013a). Few studies, however, have examined whether the temperature and desiccation conditions experienced by these animals are consistent throughout their life or change throughout ontogeny (Jenewein and Gosselin, 2013b). For young animals, at a certain point new stressors may arise, such as limited food availability, which could require ontogenetic changes in other traits, such as microhabitat use. The changes in ecological requirements and

physiological stress can concurrently result in a change in traits or a restructuring of energy allocation that may affect the intensity of temperature or desiccation experienced (Anderson et al., 2013; Pierrick et al., 2017; Vermeij, 1978). An example of these concurrent shifts in life strategy during the early benthic phase is demonstrated in the mussel *M. trossulus* as well as the snail *N. ostrina*. Both animals initially live within cryptic habitats, such as filamentous algae and mussel beds, but move to more exposed habitats later in juvenile and adult life (Gosselin, 1997; Hunt and Scheibling, 1996). Early benthic phase individuals of these species thus experience less extreme temperature and desiccation conditions than adults, and consequently early benthic phase individuals would not require the same degree of stress tolerance as adults. For species that do not change microhabitats throughout life, such as the snail *Littorina scutulata* and the crab *P. cinctipes*, tolerance to high temperatures and desiccation would remain constant throughout life.

High mortality during early life stages can have substantial consequences for population size and distribution, and for community dynamics (planktonic invertebrates: Eckert, 2008; fish: Steele, 1997; bryozoans: Yoshioka, 1982). Documenting differences in tolerance among species and life stages will help identify species that are likely to be most vulnerable to extreme weather events, and help understand the function of life strategies that are specific to each stage of life. Some studies have suggested that intertidal species are robust to climate change (Thompson et al., 2002), but such studies have not fully taken into account ontogenetic shifts in vulnerability (Byrne, 2012; Sorte et al., 2011). The present study examines six species of intertidal invertebrates that occupy different niches and thus experience a broad range of exposure to temperature and desiccation. By measuring the tolerance thresholds of these species, relationships between stress tolerance and niche dimensions can be identified. Choosing species known to undergo ontogenetic shifts in niche use and comparing them to species that retain the same niche throughout life will help elucidate the influence of changing pressures in an animals' life.

The purpose of this study was to document ontogenetic and interspecific variation in physiological tolerance to temperature and desiccation, and to examine the link between lifestyle and physiological tolerance. The specific goals of this study were to: (1) quantify ontogenetic shifts in tolerance thresholds from early benthic phase to adult phase; (2) document interspecific variation in tolerance thresholds of co-occurring intertidal

invertebrates to temperature and desiccation stress; (3) examine the role of body size in determining ontogenetic and interspecific variation in tolerance thresholds, and (4) establish the relationship between tolerance thresholds and three dimensions of the niche: intertidal height, microhabitat use, and motility.

METHODS

Study site

The study was conducted at the Bamfield Marine Sciences Centre on the west coast of Vancouver Island, Canada, using animals collected at three field sites in nearby Barkley Sound (Figure 2.1) during the summers of 2015 and 2016. Preliminary studies, used to identify the ranges and intervals of experimental conditions that would be used in definitive experiments, were carried out in 2015 and 2016. For all definitive experiments, conducted in 2016, the six species examined in this study were collected from Dixon Island (N 48°51'9", W 125°7'3"). Dixon Island is characterized by wave-exposed shores but also has small protected bays favourable to species not tolerant of high wave action.

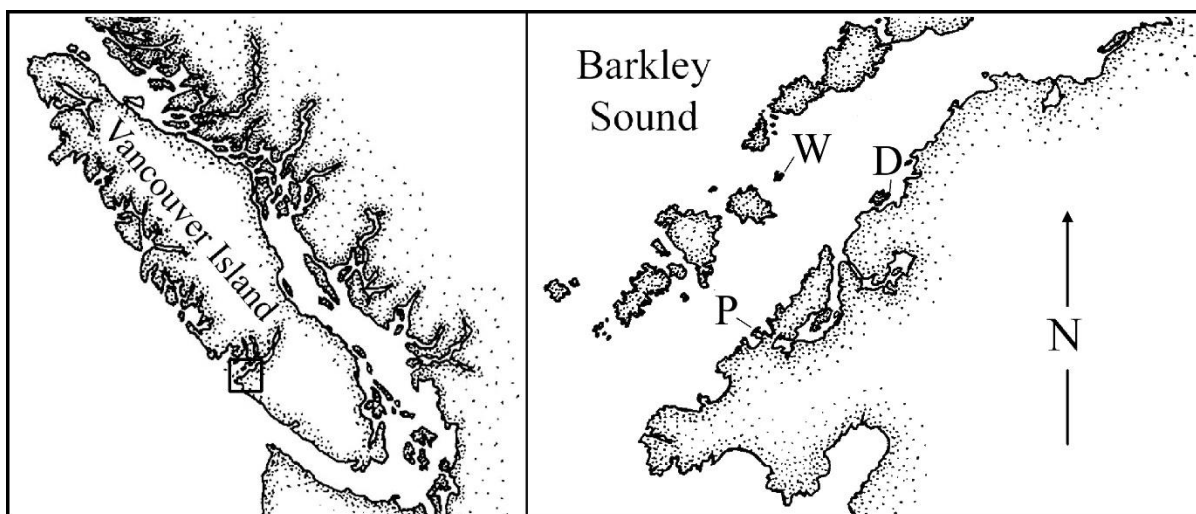


Figure 2.1. Map of field sites in Barkley Sound near Bamfield, British Columbia: Wizard Islet (W), Prasiola Point (P), Dixon (D). Map modified from Gosselin and Chia (1995).

Study organisms and collection

Six intertidal invertebrate species were chosen for this study. All species chosen for study were abundant and generally available as adults throughout the summer, and as juveniles during at least a month of each summer. *B. glandula* and *C. dalli* are barnacle species living in the mid to high intertidal zone on open, exposed surfaces; these species become sessile at metamorphosis and remain so throughout juvenile and adult life. Juveniles of the mussel, *M. trossulus*, first colonize filamentous algae where they are motile, using their foot to crawl through the microhabitat (reviewed by Hunt & Scheibling 1996); when juvenile *M. trossulus* reach 2.0-2.5 mm shell length, they attach themselves to exposed surfaces via byssal threads and remain sessile for the rest of their life (Bayne 1964, Jenewein & Gosselin 2013, Sigurdsson et al. 1976). The crab, *P. cinctipes*, occupies the low to mid intertidal range as juveniles, moving slightly higher into the mid intertidal as adults; juveniles and adults are motile and remain in cryptic microhabitats, living in the lower intertidal zone where aerial exposure periods are short (Jensen, 1989). Of the six species examined in this study, the snail *L. scutulata* occupies the highest range of the intertidal zone; both juvenile and adult *L. scutulata* are motile and live on exposed surfaces, although juveniles sometimes occupy empty barnacle shells. *N. ostrina* is a predatory snail that remains cryptic among barnacle and mussel shells as juveniles, and then venture more frequently into exposed habitats once they reach 3-8 mm shell length (Gosselin, 1997, personal observations); juvenile and adult *N. ostrina* are fully motile and occupy the mid-intertidal zone.

Temperature experiments were performed on *N. ostrina*, *M. trossulus*, *C. dalli* and *B. glandula*. For desiccation experiments, two additional species were included: *L. scutulata* and *P. cinctipes*. For adults used in temperature experiments and all size classes in desiccation experiments, *N. ostrina*, *L. scutulata*, *P. cinctipes* and *M. trossulus* were collected individually from the intertidal zone. *P. cinctipes* with autotomized claws or legs were excluded from experiments, as the integrity of the integument at the point of autotomy might be compromised, affecting desiccation tolerance. All barnacles, except for juveniles used in temperature experiments, were obtained by collecting colonized rocks (~ 10 -15 cm diameter) from the field. Individuals of each barnacle size class were marked by identifying their location on the rock with non-toxic Painters® paint markers or with Sharpie® paint markers. To collect juvenile barnacles for temperature experiments, rocks from the intertidal

zone were brought to the laboratory and all small individuals were removed with a dissection needle. A perimeter was marked around the cleared area and rocks were returned to the field for 72 h to allow the establishment of new juveniles, as in Sandee et al. (2016). Following this 3 d settlement period, the rocks were recovered and newly settled individuals were marked with a small dot on the rock surface nearby.

To collect juvenile *N. ostrina*, mature egg capsules were gently removed from rock surfaces following Gosselin and Chia (1995). Egg capsules were held in aerated seawater at 11-14°C until hatching occurred. Juvenile *M. trossulus* were extracted from the filamentous alga *Cladophora columbiana* near adult mussel beds where settlement is known to occur (Bayne, 1964), selecting recently settled juveniles measuring 250 – 700 µm shell length (Martel et al. 2000; Sandee et al. 2016). Juvenile *L. scutulata* were collected from crevices and empty barnacle shells. All animals were returned to the laboratory within 3 h of collection, where they were transferred to cages and submerged in seawater for an acclimation period of 12-24 h. *N. ostrina*, a predatory species, was held separately from the other species. The seawater used in all experiments was filtered through a 100 µm filter and all holding tanks were aerated.

Temperature tolerance

To determine tolerance thresholds to high temperatures and characterize ontogenetic trends in tolerance, juveniles and adults of four species were subjected to at least five different temperature treatments. All juveniles used in temperature tolerance experiments were ≤ 3 d old and adults were collected within the size range (Table 2.2) where individuals typically reach sexually maturity. Juvenile *C. dalli* and *B. glandula*, and adults of all species, were held in square 590 ml plastic cages with windowed lids of 400 µm mesh during the acclimation period. Following the 12-24 h acclimation period, animals were removed from these cages, were blot dried, and then transferred to an airtight 590 ml container for temperature experiments. Juvenile *M. trossulus* and *N. ostrina*, however, were held in smaller cages made by cutting the lower part of plastic microcentrifuge tubes and sealing with mesh screen (100 or 600 µm, respectively). For the temperature experiment, juvenile *M. trossulus* and *N. ostrina* remained in the microcentrifuge tube cages, which were blot-dried and then placed inside 590 ml airtight containers. Containers were then transferred for 6 h to

temperature-controlled incubators set at the experimental temperature. All containers included a seawater-saturated 4 x 4 cm paper towel to keep humidity at a high level (> 80%) and thus minimize desiccation stress.

After the 6 h temperature treatments, animals were submerged in 11-14 °C seawater for 6 h, and were then examined for survival by gently probing with a needle and observing for movement under a dissecting microscope over a 15 min period. The range of temperature treatments to which each species was exposed was selected such that mortality spanned from 0% to 100%; lower-temperature treatments in which no mortality occurred served as a control. For each species, all temperature treatments were carried out within a 2 d period in June 2016 and ranged from 24° to 60°C. For each species and life stage, the number of temperature treatments and number of replicates per treatment varied depending on availability of the animals. The experimental design of the temperature tolerance experiment for each species was therefore: 2 life stages X 5-6 temperature treatments X 3-4 replicates per temperature X 10 individuals per replicate. There was one exception to this design; due to the small number of juvenile *B. glandula* that were obtained, these were exposed to only two temperature treatments: 41 and 44 °C. Finally, the temperature at which 50% mortality was reached (LT₅₀) for each species was used as the temperature threshold for the animals being tested.

Table 2.2. Adult size ranges of the species used in temperature experiments.

Species	Body length of adults (mm)	Reference for size at maturity
<i>N. ostrina</i>	18 – 25	Lamb & Hanby 2005
<i>M. trossulus</i>	30 – 55	Kozloff & Price 1996
<i>C. dalli</i>	3 – 7	Menge 2000; Lamb & Hanby 2005
<i>B. glandula</i>	5 – 10	Barnes & Barnes 1956

Desiccation tolerance

Interspecific variation and ontogenetic trends in desiccation tolerance were examined in six species. Four size classes of each species (Table 2.3), ranging from small juvenile to full adult size, were collected for three trials in August 2016, with collections for each trial

occurring on three consecutive days prior to the trial. For each of these three trials, replicates of 10 individuals were placed in containers and the opening was covered with a coarse screen (1.3 mm mesh). The two smallest size classes of *M. trossulus*, *N. ostrina* and *L. scutulata* (classes A and B) were held in 2 ml microcentrifuge cages with widows of 100 or 600 μm mesh. The two smallest size classes of *P. cinctipes* (A and B), large size classes of *L. scutulata* (classes C and D), and juvenile *N. ostrina* in size class B were held in 40 ml cages (height: 50.8 mm, diameter; 32 mm) screened with 100 μm mesh. Large size classes of *M. trossulus*, *P. cinctipes* and *N. ostrina* (classes C and D) were held in cages screened with 200-600 μm mesh. All barnacle rocks were kept in 5.68 L cages screened with 1.3 mm mesh.

Table 2.3. Size ranges of the four size classes of species tested in the desiccation tolerance experiment.

Species	Parameter measured	Size classes (mm)			
		A	B	C	D
<i>P. cinctipes</i>	Carapace width	< 2.0	4.0 – 7.0	7.1 - 10	10.1 – 15
<i>B. glandula</i>	Shell diameter	< 2.0	2.1 – 3.0	3.1 – 5.0	7.0 – 10
<i>C. dalli</i>	Shell diameter	0.5 – 0.9	1.0 – 1.9	2.0 – 2.9	3.0 – 5.0
<i>N. ostrina</i>	Shell length	< 2.0	5.0 – 10	10.1 – 17	18 – 23
<i>M. trossulus</i>	Shell length	< 1.0	2.0 - 4.1	15 – 25	35 – 45
<i>L. scutulata</i>	Shell length	< 2.0	2. – 3.0	3.1 – 6.0	6.1- 10

Two hours before the beginning the experiment, animals were checked for mortality by gently contacting tissue with a probe and observing for movement over a 15 min period under a dissecting microscope. Immediately before animals were placed in the desiccation treatment conditions, all cages were removed from the water and blot dried. To begin the experiment, cages were placed in an incubator set at 25°C. The incubator contained two small fans placed on opposite sides of the incubator, angled towards walls to create air circulation. Replicates of each species and size class were removed after given time intervals of desiccation exposure; the time intervals ranged from 30 min to 12 h and were determined based on preliminary desiccation trials. Removal of replicates from incubators was timed to

include durations of stress exposure resulting in 0%, 100% and intermediate proportions of survival, and these durations differed among species. When removed from the desiccation treatment, animals were then immediately transferred to seawater for 6 h before examining them again for survival.

Desiccation tolerance was established by determining the relationship between survivorship and time spent in the desiccation conditions. Desiccation in this experiment was quantified using vapour pressure deficit (VPD). VPD, based on temperature and humidity measurements, is an effective measure of desiccation, as it takes into account the amount of water vapour that can be added to the air (Anderson, 1936; Jenewein and Gosselin, 2013b) and is calculated with the following equation:

$$VPD = \left(1 - \frac{RH}{100}\right) \left(610.7 * 10^{\frac{7.5T}{237.3+T}}\right)$$

VPD remained quite stable throughout each desiccation trial, and was similar among the three trials, with mean \pm standard deviation for the trials being 2.12 ± 0.22 kPa, 1.98 ± 0.22 kPa, and 2.09 ± 0.17 kPa. These desiccating conditions were comparable to the low tide conditions encountered in the rocky intertidal zone in Barkley Sound on warm summer days (Jenewein and Gosselin, 2013a). The treatment duration required to kill 50% of the individuals (LT_{d50}) was used as a measure of desiccation tolerance threshold. The experimental design of the desiccation tolerance experiment for each of the 6 species was therefore: 3 replicate desiccation trials X 4 size classes X 4-7 replicate groups per size class X 10 individuals per group.

Role of body mass in temperature and desiccation tolerance

Linear dimensions of animal body size, such as shell length, are relatively quick and easy to measure and thus were recorded for all animals used in the tolerance experiments. Body mass, however, is more relevant to an animal's sensitivity to desiccation or temperature stress than linear measurements because body mass is a more direct measure of the volume of water stored in the tissues, which in turn is a determinant of sensitivity to those stresses (Lowell, 1984). Consequently, linear measurements of body size were converted into corresponding body mass using regression equations. To determine the regression equation relating linear size to body mass for each species, a set of 40 individuals per species were

collected and the body mass and shell length or carapace width of each individual was measured (Appendix A). The relationship between body mass and temperature or desiccation tolerance was then determined using the median value of the predicted mass range of each size class and the respective LT_{50} values of each size class with a Pearson's product moment correlation. Wet mass was a better fit to body length than dry mass in regression analysis; wet body mass was therefore used to generate linear regressions for predictions of body mass for size classes of animals used in tolerance threshold experiments.

Relationship between tolerance threshold and lifestyle characteristics

To determine the relationship between tolerance thresholds and lifestyle characteristics, three lifestyle characteristics were examined for each species and life stage: intertidal height, microhabitat and motility. For analysis, two lifestyle characteristics, motility and microhabitat, were each attributed a numeric ranking based on the relative level of thermal and desiccation stress a species would encounter due to the lifestyle characteristic. A rank of 1 indicated the lowest stress, and higher numerical rank values represented higher levels of stress exposure (Table 2.4). Species living cryptically under rocks, algae or other animals are protected from exposure to stress because evaporation of water is reduced, they are sheltered from the wind, and substrate temperature is lower than on exposed surfaces (Kensler, 1967). Motile animals may encounter less thermal or desiccation stress as they are able to move to microhabitats and tidal heights that are less stressful during periods of elevated stress, whereas sessile animals are unable to change microhabitats.

For the third lifestyle characteristic, actual measurements of the upper limit of intertidal height for each species were made at three sites in Barkley Sound, within Trevor Channel. Surveys were made by searching three contiguous 5 m wide vertical sections of the intertidal zone at each site for the highest-living individual of each species. The vertical elevation of the highest individuals in each of the three sections was averaged to obtain a single value for the site; the averages from the three sites were then combined to obtain an overall average upper limit of intertidal distribution for the species. As juveniles of most species could not be found at the time of intertidal surveys, the upper limit of zonation for adults was used as a proxy measurement for juveniles. Finally, the relationships between

lifestyle characteristics and temperature or desiccation tolerance thresholds was analyzed using Pearson correlation analysis with log-transformed data ($\log_{10}(x+1)$).

Table 2.4. Definition of rankings for each lifestyle characteristic based on amount of stress expected to be encountered, where a stress ranking of 1 is lowest and stress level increases with numerical value. Information used to rank each species was obtained from the following sources: *N. ostrina*: (Gosselin, 1997); *M. trossulus*: (Bayne, 1964, pers. obs.); *P. cinctipes*: (Jensen and Armstrong, 1991); *L. scutulata*: (Kozloff and Price, 1996); *C. dalli* and *B. glandula*: (Newman and Abbott, 1980).

Characteristic	Stress Ranking				
	1	2	3	4	5
Microhabitat	Cryptic	Semi-exposed	Exposed	n/a	n/a
Motility	Motile	Motile; limited by size and water availability	Movement possible; not directional	Sessile	n/a

Data analysis

Survival data were binomial and thus did not fit a normal distribution; consequently, a generalized linear model (GLM) with a binomial error distribution and the logit link function was used to characterize the relationship between survival and either temperature treatment or time spent in the desiccation treatment, using the software R (R Core Team, 2016) (Appendix B). This method was used to fit each species and each age class for each different stressor to a separate model. To determine if a GLM adequately fits survival data, we performed goodness-of-fit tests by examining the size of the residual deviance of the model compared to its residual degrees of freedom (Faraway, 2006). These tests indicated a good fit of the model to the data for most groups. In cases where goodness-of-fit tests failed to identify a good model fit, there was evidence that lack of fit was attributed to overdispersion, which was confirmed using a bootstrapping method by Zuur et al. (2012). Two approaches were attempted to better model the overdispersed data: using a quasibinomial distribution, and adding an observation-level random effect with a different level for every observation (Harrison, 2014). Parameter estimates for each of these approaches were consistent with the original analysis. Given that GLMs are generally robust to moderate violations of

assumptions (McCulloch and Neuhaus, 2011), no measure was taken to correct for overdispersed data.

Desiccation tolerance thresholds were determined for three separate desiccation trials. Three replicate trials do not allow an accurate estimation of the random effect distribution, and thus trial was included as a fixed effect in the model (Bolker et al., 2009) when quantifying LT_{50} . In some cases, however, perfect separation occurred and a Bayesian modification of the GLM was made by specifying non-informative priors to reduce the error in parameter estimates (Gelman et al., 2008).

To identify differences among age classes of the same species, all survival data for that species for a given stressor were fit to a single GLM and this was repeated for each species and each stressor. To identify differences in survival among species or life stages for each stressor, all data were fit to a GLM including either all species of a life stage or all life stages of a single species for the temperature tolerance data and this was repeated for the desiccation tolerance data. Finally, regressions between body length and body mass were generated using log transformed data (Appendix A).

RESULTS

Temperature tolerance

Determination of tolerance

The temperature experienced by an animal was found to be a strong predictor of survival for each species and each life stage (e.g. Fig. 2.2). The relationship was best represented by a special case of the logistic regression (Appendix C):

$$P(y = 1|x) = \frac{1}{1 + e^{-(a+bx)}}$$

where a is the intercept, x is temperature, b is the temperature coefficient, and y is the proportion of individuals surviving. Based on GLM analysis, the temperature that is lethal to 50% of individuals (LT_{50}) was determined for juvenile and adult life stages of the four species (Table 2.5). The same analytical approach was also used to determine desiccation tolerance thresholds.

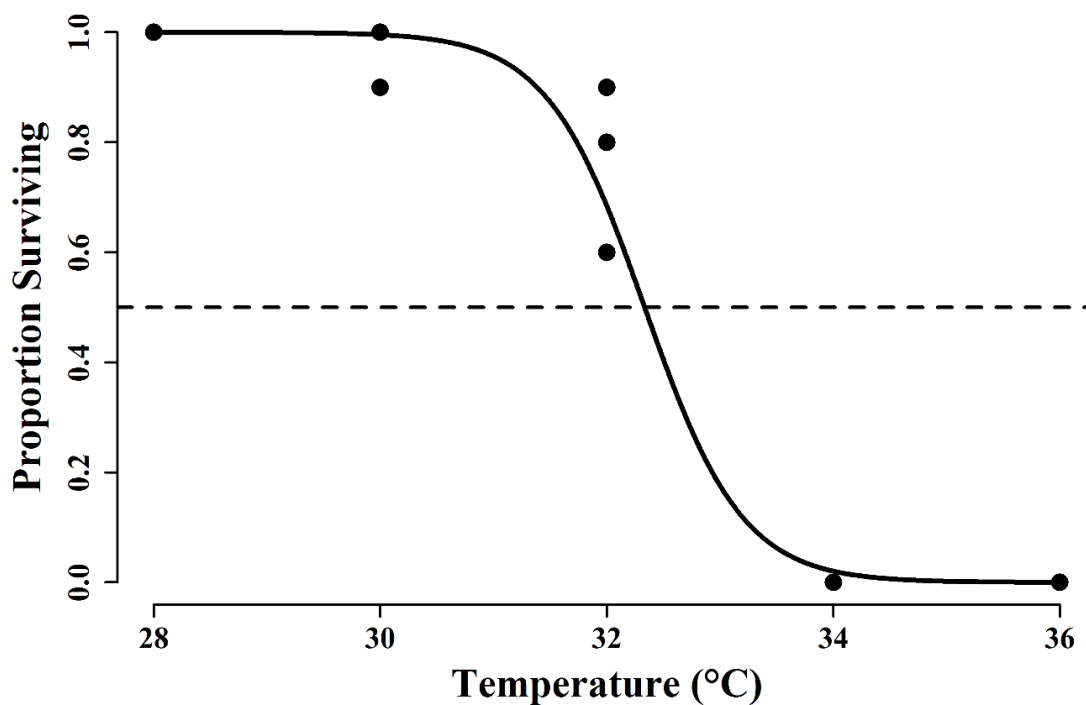


Figure 2.2. Effect of 6 h temperature treatment on the survival of *N. ostrina* juveniles where each point represents the proportion of individuals within a group of 10, surviving after a temperature treatment. A curved line is fit with estimates for the intercept and temperature parameter from a generalized linear model. A dashed horizontal line indicates LTt_{50} at the point of intersection with the fit curve.

Table 2. 5. Temperature tolerance thresholds from regressions of survival as a function of temperature (* denotes Bayesian methods were used to fit the model). The p value is for the model parameter temperature used in each GLM.

Species	Life Stage	LTt_{50} (°C)	Std. Err.	z statistic	p value	df
<i>N. ostrina</i>	Juvenile	32.3	0.14	-4.55	< 0.001	18
	Adult	32.9	0.17	-6.32	<0.001	18
<i>M. trossulus</i>	Juvenile	35.6	0.63	-6.67	<0.001	22
	Adult	* 38.3	0.18	-2.51	0.012	22
	Juvenile	45.8	0.26	-6.23	<0.001	15
<i>C. dalli</i>	Intermediate	46.0	0.23	-6.76	<0.001	18
	Adult	44.5	0.25	-7.66	<0.001	18
<i>B. glandula</i>	Adult	42.8	0.19	-6.45	<0.001	20

Ontogeny of temperature tolerance

Ontogenetic shifts in temperature tolerance were identified by combining survival data of all life stages for a single species and using a GLM to determine contrasts between life stages. The proportion of juveniles surviving at high air temperatures were significantly lower than that of adults in two species; *N. ostrina* (GLM: z-value=2.4, df=37, p=0.02) and *M. trossulus* (GLM: z-value=5.3, df=45, p<0.001) (Fig. 2.3). The proportion of *C. dalli* juveniles surviving after treatments was significantly higher than the proportion of adults surviving (GLM: z-value=3.6, df=53, p<0.001); however, adult *C. dalli* were observed to be actively mating immediately before the experiment commenced, which could have affected their tolerance limit. Therefore the temperature tolerance of an intermediate size class (1.0-2.9 mm shell diameter), slightly smaller than the reported size of adults and which likely had not yet reached sexual maturity was also tested for this species. The proportion surviving in the intermediate size class was not significantly different from juvenile survivorship (GLM: z-value=0.79, df =53, p=0.43). Thus, in all future analyses of *C. dalli*, the intermediate size class is referred to as adults.

The estimate of juvenile *B. glandula* tolerance threshold was lower than in adult *B. glandula*, but this difference could not be analyzed statistically since the juvenile tolerance threshold was based on only two temperature treatments. *M. trossulus* had the greatest change in temperature tolerance throughout life whereas *C. dalli* expressed the smallest change in tolerance throughout life (Table 2.6).

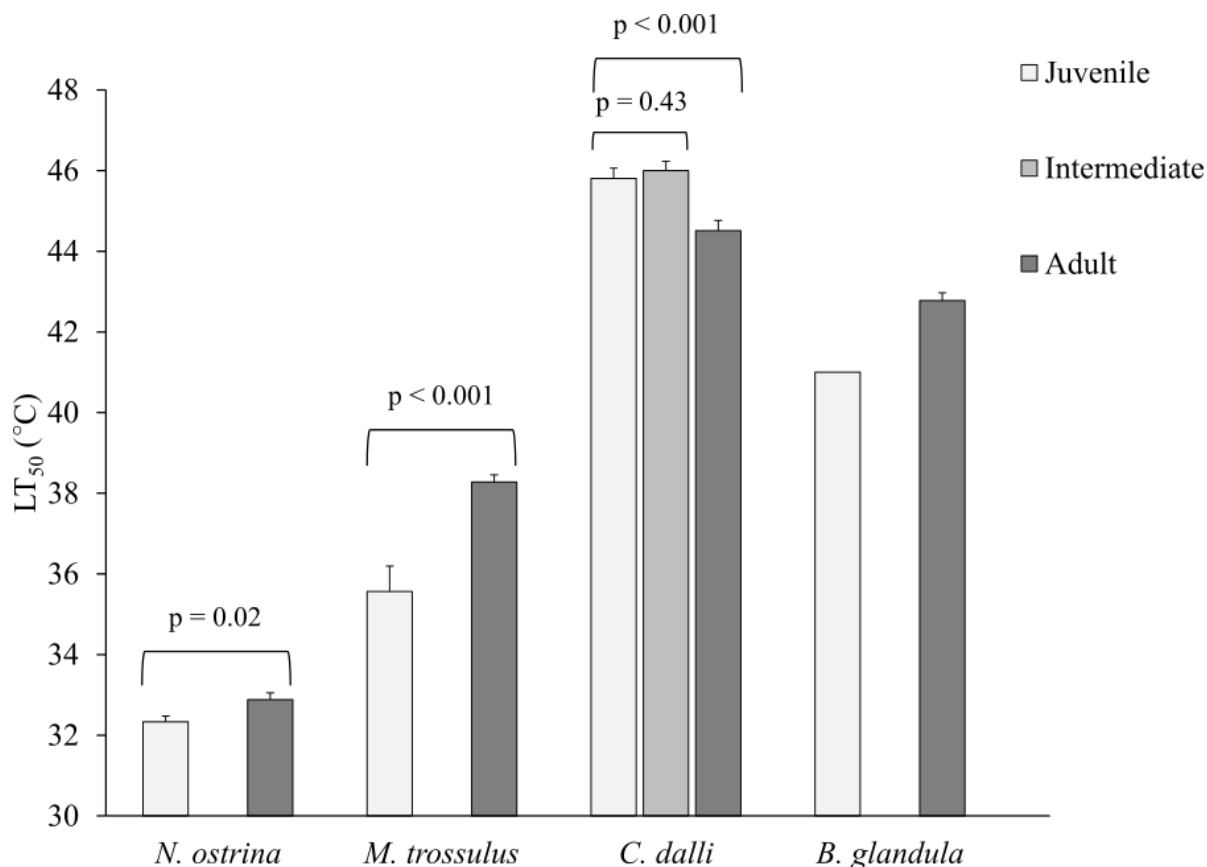


Figure 2.3. Temperature tolerance thresholds (LT₅₀ (°C)) of life stages of four species of intertidal invertebrates, as derived from logistic regressions. *B. glandula* juvenile LT₅₀ is an estimate based on two preliminary trials and was not derived from a survival curve. Significance values are derived from GLM analysis of the survival of all life stages within a species after temperature treatments. Error bars represent standard error of the LT₅₀ estimate.

Table 2.6. Difference in tolerance thresholds (LT₅₀ (°C)) between juvenile and adult size classes. Difference in *B. glandula* temperature tolerance thresholds is calculated from an estimate of juvenile tolerance.

Species	Difference in temperature tolerance between juveniles and adults (°C)
<i>B. glandula</i>	1.8
<i>C. dalli</i>	0.2
<i>M. trossulus</i>	3.3
<i>N. ostrina</i>	0.6

Interspecific variation in temperature tolerance

Interspecific variation in temperature tolerance was identified by analysing survival data from all species separately for each life stage, and comparing among species using two separate GLM with a binomial error distribution and a logit link function, one for juveniles and one for adults. The survival of each species was found to be significantly different from all other species for juveniles (GLM, pairwise contrasts among species: all z-values > 4.89, df=57, p<0.001) and adults (GLM, pairwise contrasts among species: all z-values > 9.17, df=81, p<0.001). Among juveniles and among adults, *N. ostrina* was least tolerant of high air temperatures, *M. trossulus* was the second least tolerant species, followed by *B. glandula*, and *C. dalli* (Fig. 2.3).

Desiccation Tolerance

Determination of desiccation tolerance

Following the method used to analyze temperature tolerance data, the amount of time spent in the desiccation treatment was found to significantly affect the survival of each species and life stage (e.g. Fig. 2.4) except for the largest size class of *L. scutulata*. This size class of *L. scutulata* did not reach 100% mortality even in the longest duration of exposure to desiccation (189 h) used in our trials for that species, thus preventing a stronger fit (Table 2.7).

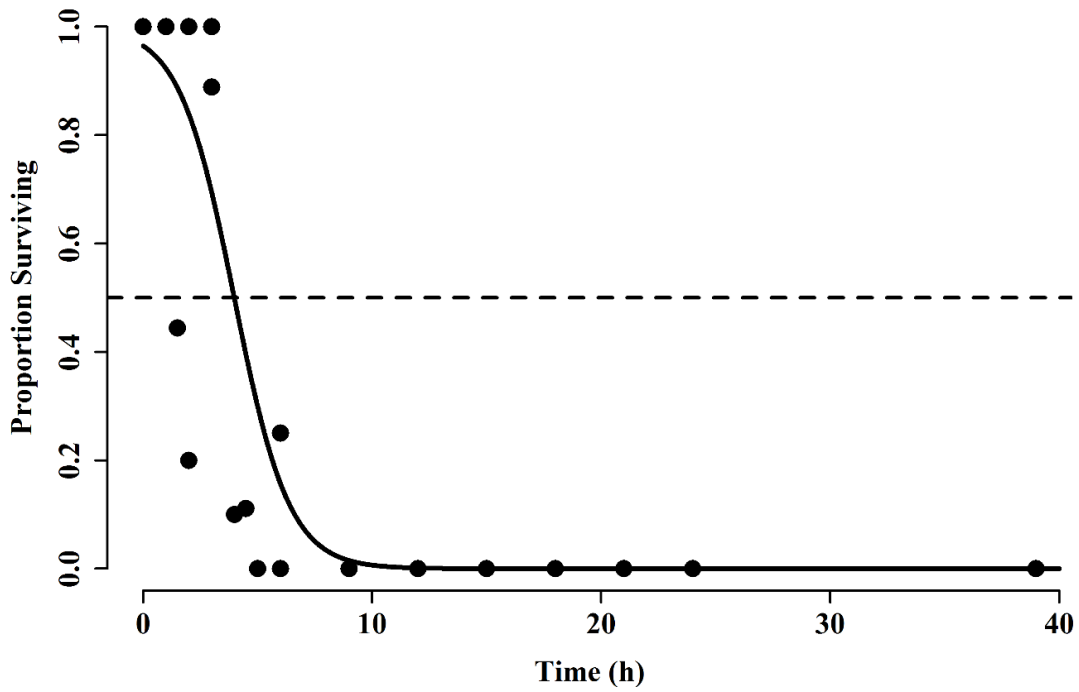


Figure 2.4. Effect of desiccation on the survival of *N. ostrina* size class A, where each point represents the proportion of individuals, within a group of 10 that survived a specific time (h) of exposure to desiccation. The curved line is fit with estimates for trial and time parameters from a generalized linear model. The horizontal line indicates LTd_{50} (h) at the point of intersection with the fit curve.

Table 2.7. Desiccation tolerance thresholds from regressions of survival as a function of time under desiccating conditions (* denotes Bayesian methods were used to fit the model). p value is for the model parameter temperature used in each GLM.

Species	Life Stage		LTd ₅₀ (h)	Std. Err.	p	df
<i>N. ostrina</i>	A	*	4.0	0.6	< 0.001	18
	B		11.7	0.7	< 0.001	13
	C		24.0	1.5	< 0.001	13
	D		34.2	2.0	< 0.001	13
<i>M. trossulus</i>	A		0.2	0.3	< 0.001	12
	B		15.3	0.9	< 0.001	13
	C		58.5	3.7	< 0.001	13
	D		52.4	3.3	< 0.001	13
<i>C. dalli</i>	A		23.0	1.2	< 0.001	15
	B		27.0	1.2	< 0.001	15
	C		42.1	2.5	< 0.001	15
	D		65.2	7.9	< 0.001	15
<i>B. glandula</i>	A		19.5	2.2	< 0.001	15
	B		32.4	2.4	< 0.001	15
	C		59.4	4.7	< 0.001	15
	D		427.5	112450.9	0.0141	15
<i>P. cinctipes</i>	A		2.60	0.3	< 0.001	10
	B	*	10.1	0.6	< 0.001	11
	C	*	7.8	0.5	< 0.001	11
	D		10.9	0.6	< 0.001	7
<i>L. scutulata</i>	A		29.0	3.7	< 0.001	13
	B		69.0	4.9	< 0.001	13
	C		142.5	10.4	< 0.001	13
	D		247.3	95.0	0.095	13

Ontogeny of desiccation tolerance

There was significant ontogenetic variation in tolerance thresholds to desiccation for each species. The smallest juvenile size class (A) was always the least tolerant to desiccation for all species (GLM, pairwise contrasts among life stages: all z-values > 2.013, df > 37, p < 0.044) (Fig. 2.5). Desiccation tolerance increased with body size across all size classes in four of the species (*N. ostrina*, *L. scutulata*, *C. dalli*, *B. glandula*), and tolerance also increased with body size across the three smallest size classes in *M. trossulus* (Fig. 2.5). In *P. cinctipes*, desiccation tolerance increased from the smallest to the next larger size class,

but then lowered for size class C and increased slightly for size class D. The magnitude of change in desiccation tolerance throughout life varied among species (Table 2.8). *C. dalli* demonstrated the smallest ontogenetic shift in desiccation tolerance from juvenile to adult stages whereas, *M. trossulus* had the greatest change in desiccation tolerance throughout life.

Table 2.8. Percent difference in desiccation tolerance thresholds between juvenile and adult size classes using LT₅₀ (h).

Species	% Difference in desiccation LT ₅₀ between size classes A and D
<i>B. glandula</i>	2,100
<i>C. dalli</i>	183
<i>L. scutulata</i>	753
<i>M. trossulus</i>	26,100
<i>N. ostrina</i>	3,020
<i>P. cinctipes</i>	319

Interspecific variation in desiccation tolerance

Interspecific variation in desiccation tolerance among newly-established juveniles (size class A) and among full-sized adults (size class D) were identified by analysing survival data of all species for a given size class and comparing species using a GLM with a binomial error distribution and a logit link function. The survivorship of newly-established juveniles in the desiccation treatment differed significantly among almost all species (GLM, pairwise contrasts among species: all z-values > 4.0, df=135, 136, p < 0.01), the only exception being between *C. dalli* and *B. glandula* (z-value= -1.08, df=135, p=0.28). Among newly-established juveniles, *L. scutulata* was the most tolerant and *M. trossulus* was the least tolerant species (Table 2.7). The survivorship of adults in the desiccation treatment also differed significantly among almost all species (GLM: all z-values > 4.8, df=136, p<0.001), the only exception being the difference between *C. dalli* and *M. trossulus* which was marginally non-significant (z-value= -1.88, df=136, p=0.06). Among adults, *B. glandula* were the most tolerant to desiccation, with a tolerance threshold of 427 h, whereas *P. cinctipes* were the least tolerant (Table 2.7).

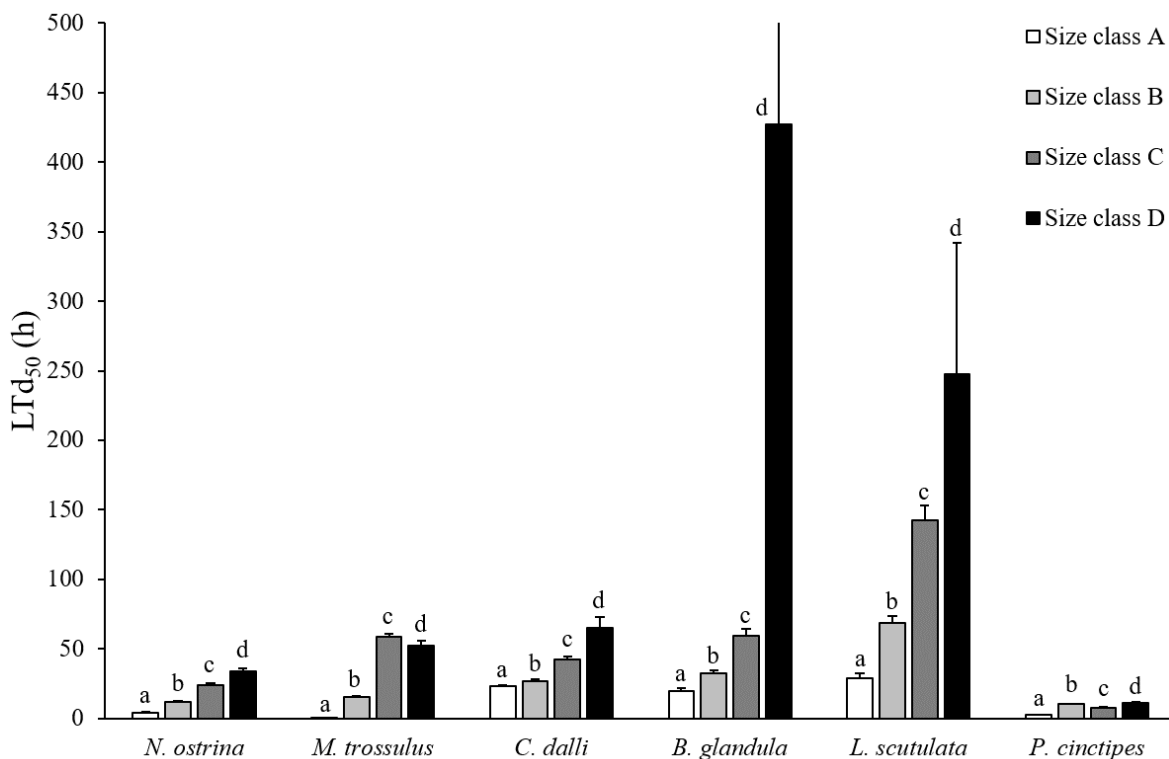


Figure 2.5. Desiccation tolerance thresholds (LT_{d50}) for six species of intertidal invertebrates as derived from logistic regressions of the survival of individuals after time at desiccating conditions. Life stage size classes represent the range of sizes available in the field for each species and do not represent the same size for all species. Different letters indicate size classes of a given species that are significantly different ($p < 0.05$) from one another, but do not apply to cross-species comparisons.

Role of body mass in temperature and desiccation tolerance thresholds

The relationship between body length and wet mass of six intertidal invertebrate species was derived using the least squares regression method. Strong correlations between body length and both dry and wet mass ($R^2 > 0.96$, $p < 0.001$, $n = 40$) were detected for each of the six species (Appendix A).

Two life stages were used in temperature experiments, precluding the analysis of the relationship between body mass and temperature tolerance within a species (i.e. ontogenetic trends). However, the relationship between body mass and temperature tolerance across species for individuals of a same life stage (i.e. interspecific trends) was analysed using a Pearson product-moment correlation coefficient; this analysis revealed no evidence of

relationship between log-transformed body mass and temperature tolerance (LT_{t50}) for either juveniles ($R^2 = 0.17$, $n = 4$, $p = 0.59$) or adults ($R^2 = 0.56$, $n = 4$, $p = 0.25$).

Body mass was strongly correlated to desiccation tolerance through ontogeny for four of the species (log-transformed tolerance threshold and mass data; *N. ostrina*: $R^2=0.99$, $n=4$, $p=0.011$; *B. glandula*: $R^2=0.94$, $n=4$, $p=0.028$; *L. scutulata*: $R^2=0.98$, $n=4$, $p=0.007$; *C. dalli*: $R^2=0.92$, $n=4$, $p=0.042$). The correlation, however, was marginally non-significant for two species (*M. trossulus*: $R^2=0.84$, $n=4$, $p=0.086$; *P. cinctipes*: $R^2=0.86$, $n=4$, $p=0.071$).

The relationship between mass and desiccation tolerance was further investigated by inspecting the trend in the relationship between LTD_{50} (h) and body mass (g). Tolerance thresholds in four of the species increased with body size through all four size classes (Fig. 2.6). *P. cinctipes* and *M. trossulus* did not follow this trend; rather, desiccation tolerance levelled off at late juvenile or adult body sizes. The relationship between mass and desiccation tolerance threshold appeared to be asymptotic in most species, with an initial sharp increase in tolerance among small size classes followed by a gradual levelling off at larger size classes. With regards to interspecific variation in desiccation tolerance, body mass was not significantly related to desiccation tolerance thresholds among newly established juveniles or among adults (Pearson product-moment correlation coefficient analyses; class A: $R^2 = 0.47$, $n = 6$, $p = 0.68$; class D: $R^2 = 0.23$, $n = 6$, $p = 0.33$).

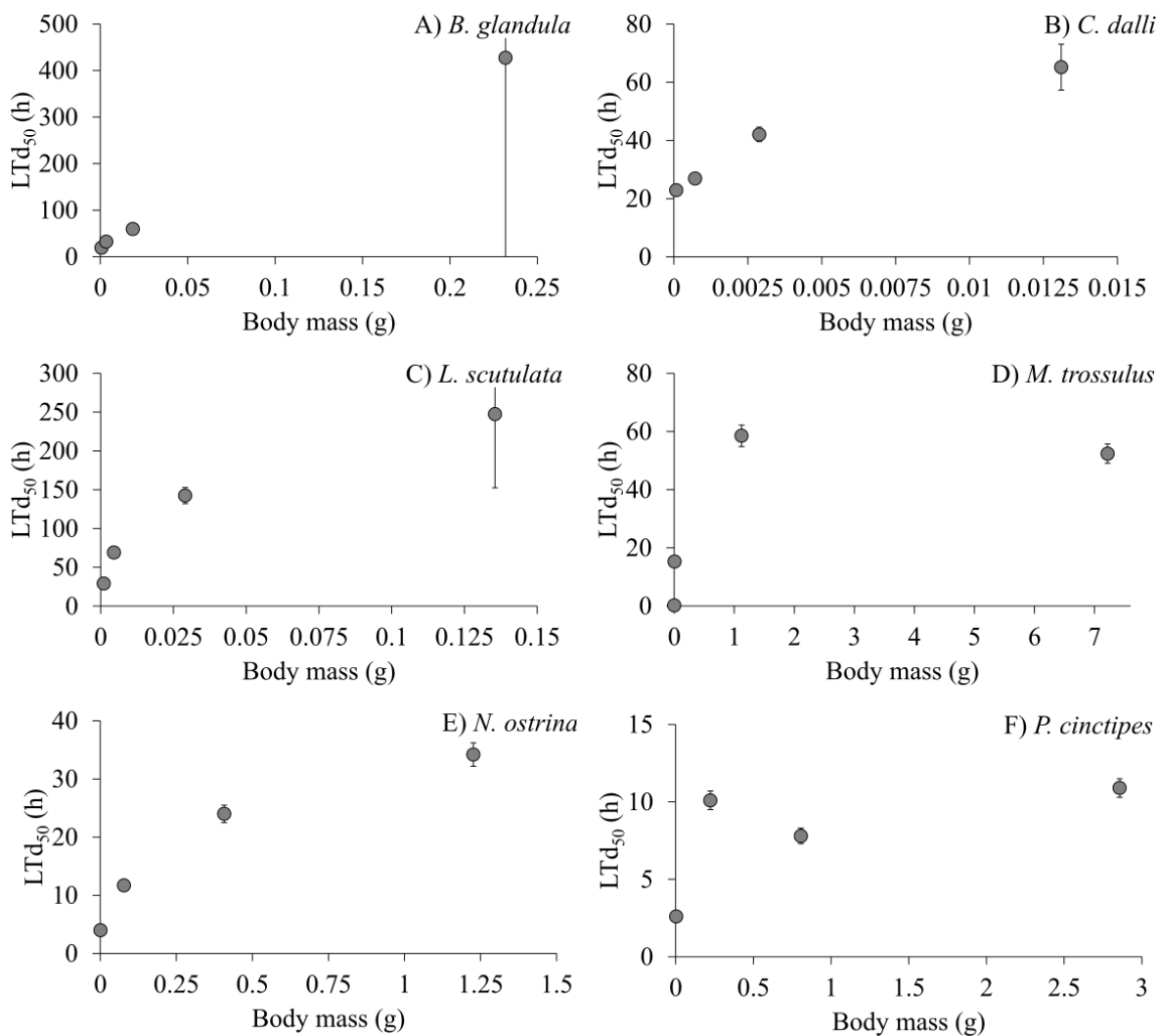


Figure 2.6. Desiccation tolerance thresholds (LTd₅₀) as a function of body mass (g) for six intertidal invertebrate species (A: *B. glandula*, B: *C. dalli*, C: *L. scutulata*, D: *M. trossulus*, E: *N. ostrina*, F: *P. cinctipes*).

Relationship between tolerance threshold and lifestyle characteristics

To identify lifestyle characteristics that play a role in an animals' tolerance to temperature or desiccation, three characteristics were assessed for each species at juvenile and adult life stages: upper limit of intertidal range, microhabitat and motility. In addition, Pearson's product-moment correlation coefficients were calculated for each pair of characteristics (log-transformed) to identify relationships between characteristics and temperature or desiccation tolerance thresholds (Appendix D).

In juveniles, a large proportion of the interspecific variation in temperature tolerance was explained by the upper limit of intertidal range of the species (log-transformed data: $R^2 = 0.86$, $n=4$, $p = 0.070$), motility ranking ($R^2 = 0.85$, $n=4$, $p = 0.078$), as well as microhabitat ranking ($R^2 = 0.85$, $n=4$, $p = 0.078$), although those relationships were not quite significant, likely due to small sample sizes ($n=4$ species). There was also a high degree of covariance between intertidal height, microhabitat ranking and motility ranking for juveniles, but the importance of this covariance in correlation analyses is difficult to assess as there were only 3-5 levels of each lifestyle characteristic and four species used in the analysis. In adults, temperature tolerance thresholds were not significantly related to microhabitat use or to motility ranking, but did appear to be related to the upper limit of intertidal zonation (log-transformed data: $R^2 = 0.87$, $n=4$, $p = 0.068$) (Fig. 2.7).

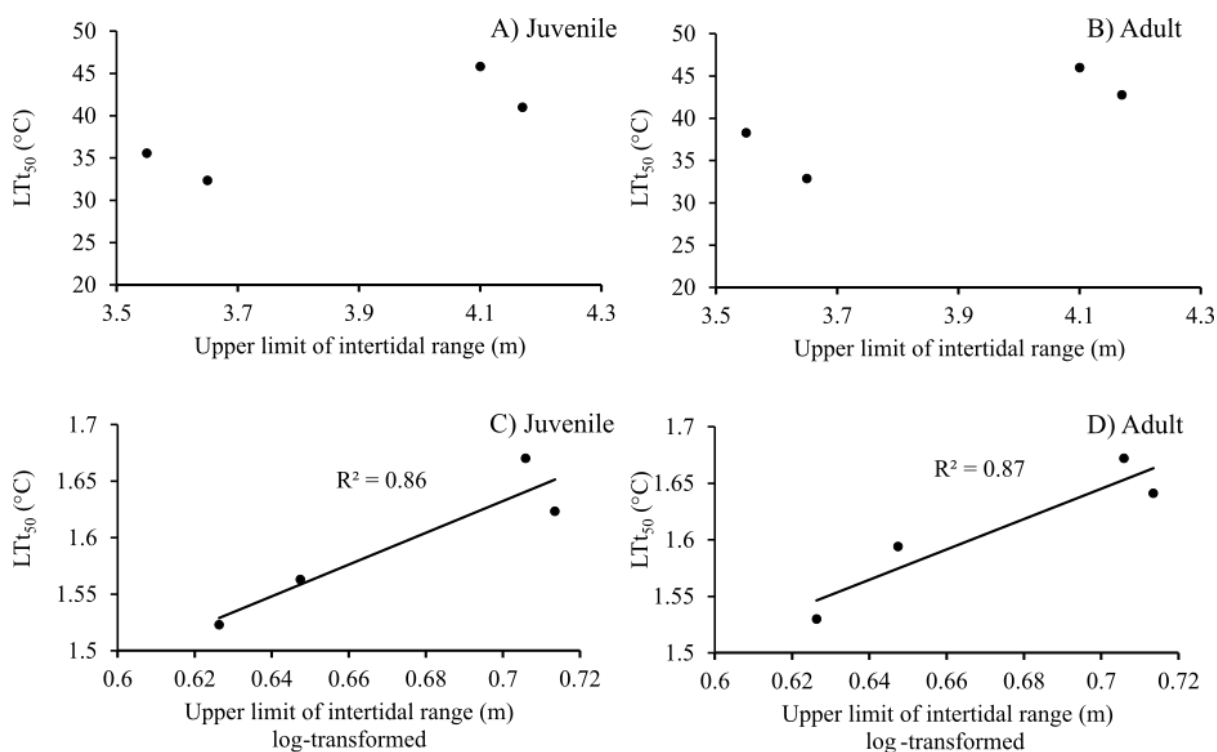


Figure 2.7. Relationship between temperature tolerance (LTt₅₀) and upper limit of intertidal range among juvenile and adult intertidal invertebrates.

Interspecific variation in desiccation tolerance in newly established juveniles (size class A) was largely explained by the intertidal height of the species ($R^2 = 0.62$, $p = 0.060$, $n=6$), although the relationship was marginally non-significant. Desiccation tolerance in

juveniles was also related to microhabitat ranking ($R^2 = 0.72$, $p = 0.030$, $n = 6$), but not to motility ranking ($p=0.58$).

In adults, interspecific variation in desiccation tolerance thresholds was significantly related to the upper limit of intertidal zonation (log-transformed data: $R^2 = 0.77$, $n = 6$, $p = 0.020$) (Fig. 2.8). There was also a likely relationship between desiccation tolerance threshold and microhabitat ranking ($R^2 = 0.62$, $n = 6$, $p = 0.060$), but not between desiccation tolerance thresholds and motility ranking ($p=0.24$). All correlation analyses suggest a relationship between intertidal height and stress tolerance for both juveniles and adults, as well as a link between stress tolerance and microhabitat use.

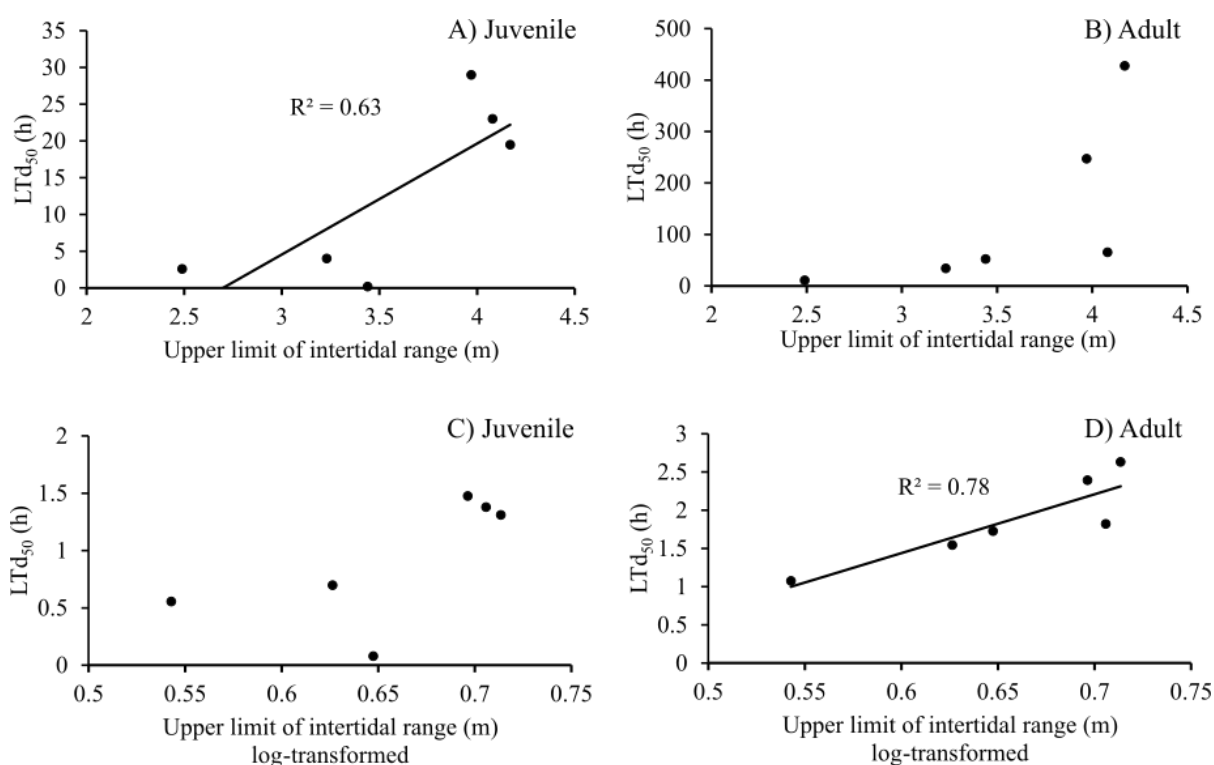


Figure 2.8. Relationship between desiccation tolerance (LTd₅₀) and upper limit of intertidal range among juvenile and adult intertidal invertebrate.

DISCUSSION

Ontogenetic variation in stress tolerance

Temperature tolerance thresholds increased significantly throughout ontogeny in the three species that could be analyzed; the increases were relatively modest, with LT₅₀ values only increasing by 0.6 – 2.7°C. This finding nevertheless reveals that high temperatures are a greater threat for early benthic phase individuals than for adults. Desiccation tolerance thresholds also changed throughout ontogeny, with LT₅₀ values increasing by at least 182% in *C. dalli*, and increasing by 26,100% in *M. trossulus*. These considerable shifts in desiccation tolerance occurred progressively throughout ontogeny in four of the species; in two species (*M. trossulus* and *P. cinctipes*), however, desiccation tolerance increased rapidly among the smallest size classes but then levelled off among larger sizes. Additionally, the magnitude of ontogenetic changes in desiccation tolerance between juvenile and adult stages was greater for all species than the changes in temperature tolerance throughout life such that desiccation is likely a more important pressure on juveniles than high temperature.

The tolerance thresholds of small juvenile invertebrates to elevated temperature and to desiccation were lower than the tolerance thresholds of adults in almost all species, indicating that significant ontogenetic shifts in physiological tolerance to these stressors are common. In our experiments, *C. dalli* was the only exception to this trend, with temperature tolerance thresholds of newly settled juveniles and of adults being nearly identical and with the smallest ontogenetic shift in desiccation tolerance among the 6 species.

Research has sought to explain causes for early post-settlement mortality of benthic marine invertebrates which decreases dramatically with age (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Evidence of ontogenetic shifts in body colouration, microhabitat use, desiccation tolerance and vulnerability to predators provide a partial explanation of early benthic phase mortality which tapers off with age (Anderson et al., 2013; De Bruyn and Gosselin, 2014; Gosselin, 1997; Gosselin and Jones, 2010; Snover, 2008). However, there has been little focus on the physiological stress tolerance of individuals during the early benthic phase and the direct role of intertidal temperatures and desiccation on tolerance thresholds. As identified by this study, early benthic phase tolerance thresholds to high temperature and desiccation would not allow those individuals to survive the full range of plausible environmental conditions characteristic of Barkley Sound. Adult temperature and

desiccation tolerance thresholds, however, often exceeded the range of conditions occurring in the field, thus highlighting the changes in vulnerability to low tide stress throughout life. These ontogenetic shifts in vulnerability to high temperatures and desiccation could contribute to a multi-faceted explanation of high early benthic phase mortality followed by greatly decreasing mortality.

Interspecific variation in stress tolerance

The temperature tolerance thresholds of early benthic phase individuals differed significantly among species, ranging from 32.3°C (*N. ostrina*) to 45.8°C (*C. dalli*). Given that intertidal temperatures in Barkley Sound can reach 45°C, juvenile tolerance thresholds of all species, except *C. dalli*, were lower than the temperature maxima expected in this region and thus are susceptible to mortality from high temperatures. Temperature tolerance thresholds of adults also differed significantly among species, ranging from 32.9°C (*N. ostrina*) to 46.0°C (*C. dalli*). Again, *C. dalli* was the only species whose adult tolerance threshold exceeded the full range of expected environmental conditions, indicating these adults may not be susceptible to mortality caused by high temperatures in that region.

When exposed to moderately-high desiccation levels (VPD=2.06 kPa), desiccation tolerance thresholds for early benthic phase individuals ranged from 10 min (*M. trossulus*) to 29 h (*L. scutulata*). By comparison, VPD levels monitored on intertidal rock surfaces at low tide in Barkley Sound (Jenewein and Gosselin, 2013b) fluctuated considerably throughout the summer, with daily VPD levels often exceeding the levels used in the present experiment, and reaching levels as high as 6.85 kPa. Given the low desiccation tolerance thresholds of juveniles of three of the six species studied (*M. trossulus*, *P. cinctipes* and *N. ostrina*), new recruits of these species would be highly vulnerable to ambient desiccating conditions occurring in the intertidal zone throughout the summer months. Adults were much more tolerant of those same desiccating conditions, with LT₅₀ values ranging from 10.8 (*P. cinctipes*) to 427 h (*B. glandula*). The lowest LT₅₀ for adult desiccation tolerance threshold (*P. cinctipes*) exceeded their aerial exposure time during a tidal cycle (~ 8 h); this suggests they could survive direct exposure to desiccation conditions in the field on most days, although they would likely remain vulnerable to the most extreme desiccating conditions. Adults of the five other species all had desiccation tolerance thresholds exceeding 34 h; these

were all highly tolerant of desiccation and would likely survive most, if not all, desiccation conditions experienced in the field.

There was also interspecific variation in the magnitude of ontogenetic shifts of desiccation tolerance. The greatest ontogenetic shifts in temperature tolerance threshold occurred in *M. trossulus* (3.3°C), compared to only 0.2°C in *C. dalli*. Ontogenetic shifts in desiccation tolerance differed even more among species, ranging from 183% in *N. ostrina* to 26,100% in *M. trossulus*.

Role of body mass in temperature and desiccation tolerance

Ontogenetic differences in desiccation tolerance were explained, at least in part, by differences in body mass among size classes. The relationship between desiccation tolerance and body mass is unsurprising, as surface area to volume ratios, which are greater in smaller animals, impact the rate of evaporation, and desiccation tolerance is largely affected by evaporation rate (Lowell, 1984). In desiccation experiments, tolerance in a given species increased with body mass in a logarithmic manner, increasing rapidly among smaller mass size classes and then more slowly among larger size classes (Fig. 2.6). The shape of this relationship resembles that of the relationship between volume and the surface area to volume ratio of a three dimensional object (Appendix C), suggesting that ontogenetic increases in tolerance are largely a consequence of corresponding changes in surface to volume ratio. Body mass, however, did not explain interspecific variation in desiccation tolerance. For example, adults of the two barnacle species were the most tolerant to desiccation of all species examined in this study, yet they also had the smallest adult size. These findings indicate that other traits, such as behavioural, anatomical or physiological differences, must be responsible for the interspecific differences in temperature and desiccation tolerance.

Relationship between tolerance thresholds and lifestyle characteristics

Juvenile and adult temperature and desiccation tolerance increased with the upper limit of intertidal distribution. Although correlations with intertidal distribution were marginally non-significant except in adult desiccation tolerance, it appears that animals are

either limited in their intertidal range by tolerance or evolve a tolerance to meet a certain upper limit of vertical distribution.

Juvenile tolerance to temperature and desiccation was also related to microhabitat use, although the relationships were marginally non-significant. Species occupying exposed microhabitats demonstrated greater tolerance thresholds to high temperatures and desiccating conditions than animals living cryptically. This finding is consistent with previous work by Kensler (1967) indicating that high temperature and desiccation stress are related to the use of crevices as cryptic microhabitats.

Juvenile temperature tolerance was typically greater for sessile animals. Motility ranking and juvenile tolerance thresholds were not related, nor was motility rankings of adults related to temperature or desiccation tolerance. This is surprising, as the difference in lifestyle associated with a motile or sessile strategy is dramatic among species and correlates to the amount of temperature or desiccation stress an animal may experience. It is notable that *M. trossulus* exhibited the greatest shift in tolerance to temperature and desiccation, as this species also undergoes significant ontogenetic shifts in motility and microhabitat use. *C. dalli* and *B. glandula* typically had small changes in tolerance thresholds to temperature and desiccation. Since neither of these species experience substantial ontogenetic shifts in motility or microhabitat use, it is conceivable that tolerance would not change much throughout life either. There was, however, a high degree of covariance between microhabitat ranks and motility ranks thus it is difficult to identify which lifestyle characteristic is most influential to tolerance thresholds.

As desiccation is related to ambient temperature, it is likely that covariation also occurs between the two stressors that were tested. In these experiments, temperature was kept at a non-lethal level during desiccation experiments to minimize the effect of temperature on mortality. However, temperature has been shown to interact with a number of stressors in the marine environment to amplify mortality and thus temperature and desiccation stress may act in a compounding manner here (Miller and Waldbusser, 2016; Sokolova et al., 2012). These experiments did not investigate the interaction between high temperature and desiccation stress on tolerance thresholds, though these findings would be insightful since these two stressors likely vary together in the natural environment.

The positive relationship between desiccation tolerance and microhabitat ranking in early benthic phase individuals points to the phenomenon of early juvenile mortality first identified in a study by Gosselin & Qian (1996) and later reviewed by Gosselin & Qian (1997) and Hunt & Scheibling (1997). These studies revealed that death in the early benthic phase can exceed 90% for the majority of species. If juveniles are particularly vulnerable to high desiccation because of a reduced physiological tolerance, they may have need to use different microhabitats relative to their adult conspecifics. Conversely, if these protected habitats are advantageous for other unknown reasons, high physiological tolerance need not have evolved in early ontogeny. Since adult temperature tolerance is not correlated with microhabitat use, it appears adults are not limited in the same manner by exposure as juveniles.

The three niche dimensions described here, while not comprehensive, represent differences among species that are important in determining physiological stress tolerance. Tolerance to temperature and to desiccation stress were positively related to intertidal height, suggesting that these stress tolerance and upper limit of intertidal range may have evolved concurrently. Since only juveniles demonstrated significant relationships between microhabitat and temperature tolerance, where an animal lives then, can provide information about the physiological tolerance thresholds to low tide stress for juveniles but might not be as important for adults.

Evolutionary and ecological implications

Ontogenetic shifts in temperature tolerance and desiccation tolerance were observed in all species tested in this study. A consequence of these ontogenetic shifts is that the threats to survival change throughout life. With increased understanding of the periods of life when animals are most vulnerable to environmental stressors, population fluctuations that are common among benthic invertebrates can be better predicted. Thus, future studies should aim to identify vulnerable periods in an animal's life to better inform conservation efforts and predictive abilities of those populations studied, through multiple life stage experiments.

Since instances of extreme weather events are increasing and air temperatures in British Columbia are warming (Lemmen et. al, 2016), understanding the tolerance thresholds of all species is essential in predicting how intertidal communities will be affected by these

changes in climate. The widespread interspecific differences in temperature and desiccation tolerance thresholds identified by my study, illustrates that future research should aim to identify a representative species to set future policy and regulations for the protection of intertidal species in the face of changing conditions.

LITERATURE CITED

Anderson DB (1936) Relative Humidity or Vapor Pressure Deficit. *Ecol Soc Am* 17:277–282

Anderson JR, Spadaro AJ, Baeza JA, Behringer DC (2013) Ontogenetic shifts in resource allocation: Colour change and allometric growth of defensive and reproductive structures in the Caribbean spiny lobster *Panulirus argus*. *Biol J Linn Soc* 108:87–98

Barnes H, Barnes M (1956) The General Biology of *Balanus glandula* Darwin. *Pacific Sci* 10:415–422

Bayne BL (1964) Primary and Secondary Settlement in *Mytilus edulis* L. (Mollusca). *J Anim Ecol* 33:513–523

Bertness MD, Leonard GH, Levine JM, Bruno JF (1999) Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia* 120:446–450

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135

Bruyn R a J De, Gosselin LA (2014) Prevalence of ontogenetic changes in colour brightness among benthic invertebrates and their association with microhabitat shifts. *Mar Ecol Prog Ser* 498:147–159

Byrne M (2012) Global change ecotoxicology: Identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Mar Environ Res* 76:3–15

- Eckert GL (2008) Effects of the Planktonic Period on Marine Population Fluctuations. *Ecol Soc Am* 84:372–383
- Faraway JJ (2006) Linear Models with R and Extending the Linear Model with R. *J Stat Softw* 17:1–11
- Gelman A, Jakulin A, Pittau MG, Su Y-S (2008) A Weakly Informative Default Prior Distribution for Logistic and Other Regression Models Author (s): Andrew Gelman , Aleks Jakulin , Maria Grazia Pittau and Yu-Sung Su Source : The Annals of Applied Statistics , Vol . 2 , No . 4 (Dec . , 2008), pp . 13. *Ann Appl Stat* 2:1360–1383
- Gosselin LA (1997) An ecological transition during juvenile life in a marine snail. *Mar Ecol Prog Ser* 157:185–194
- Gosselin LA, Chia FS (1995) Characterizing temperate rocky shores from the perspective of an early juvenile snail: the main threats to survival of newly hatched *Nucella emarginata*. *Mar Biol* 122:625–635
- Gosselin LA, Jones LA (2010) Effects of solar radiation on barnacle settlement, early post-settlement mortality and community development in the intertidal zone. *Mar Ecol Prog Ser* 407:149–158
- Gosselin LA, Qian PY (1996) Early post-settlement mortality of an intertidal barnacle: A critical period for survival. *Mar Ecol Prog Ser* 135:69–75
- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- Harrison XA (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2:e616
- Hayward S a L, Rinehart JP, Denlinger DL (2004) Desiccation and rehydration elicit distinct heat shock protein transcript responses in flesh fly pupae. *J Exp Biol* 207:963–971
- Hunt HL, Scheibling RE (1996) Physical and biological factors influencing mussel (*Mytilus trossulus*, *M. edulis*) settlement on a wave-exposed rocky shore. *Mar Ecol Prog Ser* 142:135–145

- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar Ecol Prog Ser* 155:269–301
- Jenewein BT, Gosselin LA (2013a) Early benthic phase mortality of the barnacle *Balanus glandula* is influenced by *Fucus* spp. cover but not by weather-related abiotic conditions. *J Exp Mar Bio Ecol* 449:28–35
- Jenewein BT, Gosselin LA (2013b) Ontogenetic shift in stress tolerance thresholds of *Mytilus trossulus*: Effects of desiccation and heat on juvenile mortality. *Mar Ecol Prog Ser* 481:147–159
- Jensen GC (1989) Gregarious settlement by megalopae of the porcelain crabs *Petrolisthes cinctipes* (Randall) and *P. eriomerus* Stimpson. *J Exp Mar Bio Ecol* 131:223–231
- Jensen GC, Armstrong DA (1991) Intertidal zonation among congeners: factors regulating distribution of porcelain crabs *Petrolisthes* spp. (Anomura: Porcellanidae). *Mar Ecol Prog Ser* 73:47–60
- Kensler CB (1967) Desiccation resistance of intertidal crevice species as a factor in their zonation. *J Anim Ecol* 36:391–406
- Kozloff EN, Price LH (1996) *Marine invertebrates of the Pacific Northwest*. University of Washington Press
- Lamb A, Hanby BP (2005) *Marine Life of the Pacific Northwest (A Photographic Encyclopedia of Invertebrates, Seaweeds and Selected Fishes)*.
- Lemmen DS, Warren FJ, James TS, Mercer Clarke, C.S.L editors (2016): *Canada's Marine Coasts in a Changing Climate*; Government of Canada, Ottawa ON
- Lowell RB (1984) Desiccation of intertidal limpets: Effects of shell size, fit to substratum, and shape. *J Exp Mar Bio Ecol* 77:197–207
- Marsden ID, Weatherhead MA (1998) Effects of aerial exposure on oxygen consumption by the New Zealand mussel *Perna canaliculus* (Gmelin, 1791) from an intertidal habitat. *J Exp Mar Bio Ecol* 230:15–29

- Martel AL, Auffrey LM, Robles CD, Honda BM (2000) Identification of settling and early postlarval stages of mussels (*Mytilus* spp.) from the Pacific coast of North America, using prodissoconch morphology and genomic DNA. *Mar Biol* 137:811–818
- McCulloch C, Neuhaus J (2011) Prediction of random effects in linear and generalized linear models under model misspecification. *Biometrics* 67:270–279
- Menge BA (2000) Recruitment vs. postrecruitment processes as determinants of barnacle population abundance. *Ecol Monogr* 70:265–288
- Miller LP, Harley CDG, Denny MW (2009) The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea*. *Funct Ecol* 23:756–767
- Miller C, Waldbusser G (2016) A post-larval stage-based model of hard clam *Mercenaria mercenaria* development in response to multiple stressors: temperature and acidification severity. *Mar Ecol Prog Ser* 558:35–49
- Mislan KAS, Helmuth B, Wetthey DS (2014) Geographical variation in climatic sensitivity of intertidal mussel zonation. *Glob Ecol Biogeogr* 23:744–756
- Newman W a., Abbott DP (1980) Cirripedia: the barnacles. In: *Intertidal Invertebrates of California*. Stanford University Press, Stanford.p 504–535
- Pierrick B, Tarik M, Martin F, Réjean T, René R, Frédéric O (2017) Nursery function of coastal temperate benthic habitats: New insight from the bivalve recruitment perspective. *J Sea Res* 121:11–23
- Sandee S, Hamme J Van, Gosselin L (2016) Testing microbial pathogens as a cause of early juvenile mortality in wild populations of benthic invertebrates. *Mar Ecol Prog Ser* 562:53–63
- Sigurdsson JB, Titman CW, Davies PA (1976) The dispersal of young post-larval bivalve molluscs by byssus threads. *Nature* 262:386–387
- Snover ML (2008) Ontogenetic habitat shifts in marine organisms: Influencing factors and the impact of climate variability. *Bull Mar Sci* 83:53–67

- Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integr Comp Biol* 53:597–608
- Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar Environ Res* 79:1–15
- Somero GN (2002) Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integr Comp Biol* 42:780–789
- Sorte CJB, Jones SJ, Miller LP (2011) Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *J Exp Mar Bio Ecol* 400:209–217
- Steele M a. (1997) Population regulation by post-settlement mortality in two temperate reef fishes. *Oecologia* 112:64–74
- Stillman J, Somero G (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. *J Exp Biol* 199:1845–1855
- Thompson R, Crowe T, Hawkins S (2002) Rocky intertidal communities: Past environmental changes, present status and predictions for the next 25 years. *Environ Conserv* 29:168–191
- Tomanek L, Helmuth B (2002) Physiological Ecology of Rocky Intertidal Organisms: A Synergy of Concepts. *Integr Comp Biol* 42:771–775
- Vermeij GJ (1978) *Biogeography and Adaptation: Patterns of Marine Life*. Harvard University Press
- Wolcott G (1973) Physiological ecology and intertidal zonation in limpets (*Acmaea*): A critical look at “Limiting Factors.” *Biol Bull* 145:389–482
- Yoshioka PM (1982) Role of Planktonic and Benthic Factors in the Population Dynamics of the Bryozoan *Membranipora Membranacea*. *Ecology* 63:457–468

Zuur AF, Saveliev AA, Ieno EN (2012) Zero inflated models and generalized linear mixed models with R. Highland Statistics Ltd

CHAPTER 3: General conclusion

Summary of results

To better understand the ontogeny of tolerance to temperature and desiccation stress in intertidal invertebrates, this study (1) documented interspecific variation in tolerance thresholds of intertidal invertebrates to elevated air temperature and desiccation stress; (2) quantified ontogenetic shifts in tolerance thresholds from early benthic phase to adult phase; and (3) established the relationship between tolerance thresholds and motility, microhabitat use and intertidal height of these species. The highlights of this study are as follows: (1) there was extensive interspecific variation in tolerance to low tide stress of the species tested; tolerance thresholds to high air temperatures ranged from 32.3 to 46.0 °C and tolerance thresholds to desiccating conditions ranged from 10 min to 427 h; (2) ontogenetic shifts in air temperature and desiccation tolerance occur for all species tested ranging from changes of 0.4 to 26,100 % difference; and (3) motility was not significantly correlated to juvenile or adult tolerance, but stress due to intertidal range and microhabitat were correlated to tolerance thresholds in juveniles and adults.

The widespread interspecific variation in temperature and desiccation tolerance that was observed in these experiments highlights the differences in energetic requirements and lifestyle needs of each species. Animals that are closely related and those sharing the same ecosystem are evidently very different from one another if tolerance limits vary so dramatically among species. The mechanisms for tolerance to the high temperatures and desiccation experienced by intertidal invertebrates at low tide are extensive and diverse, so differences among species in tolerance thresholds was expected. There were, however, some similarities in interspecific trends of temperature and desiccation tolerance thresholds. Species that were most tolerant of high temperatures, *C. dalli* and *B. glandula*, were also among the most tolerant to desiccation. This points to similarities in these types of low tide stress, as it is known that both elicit similar physiological mechanisms for tolerance.

The magnitude of ontogenetic shifts in temperature and desiccation tolerance thresholds differed considerably. Ontogenetic changes in temperature tolerance were modest in all species. Changes in desiccation tolerance thresholds on the other hand, were large and often adults were more tolerant than might be expected, given the desiccation conditions that

are known to occur in Barkley Sound. Juvenile temperature tolerance thresholds were within the plausible range of environmental conditions in Barkley Sound and desiccation tolerance thresholds of some species were within the duration typical of low tide. Therefore both high temperature and desiccation can be expected to impact population dynamics and community structure, particularly through effects on juveniles. The occurrence of ontogenetic shifts in tolerance was consistent for all species, thus further enforcing findings of previous studies which have identified the early benthic phase as a distinct ecological phase for marine invertebrates (Gosselin and Qian, 1997; Hunt and Scheibling, 1997).

Finally, it appears that motility is not significantly correlated to desiccation tolerance thresholds for juveniles or adults of the species that were tested; and was only correlated to temperature tolerance thresholds in juveniles. Two other dimensions of the niche were nevertheless significantly related to juvenile temperature and desiccation tolerance: microhabitat use and upper range of intertidal distribution. The relationship between microhabitat and tolerance are weaker in adults, however, suggesting possible differences in selective pressure between juveniles and adults. The relationships in juveniles between tolerance thresholds, microhabitat use and intertidal distribution indicates that animals may either alter their tolerance to suit their niche or, alternatively, choose their niche based on a predetermined tolerance.

Relevance of findings for policy

The findings of this study are relevant to two areas of Canadian policy: (1) British Columbia regulations on the protection of the intertidal zone, and (2) climate change policy. Quantifying the degree to which species living side by side differ in their tolerance to stress is essential to understanding changes in a community. This study shows that species will respond differently to periods of high stress; if these differences in tolerance result in differential mortality in nature, the difference will likely impact population dynamics as well as community structure.

In British Columbia's regulation of intertidal zones, there is limited legally binding policy for restricting coastal development. Environmental assessments are required only if development spans more than 1 km of coastline or 2 ha of seabed, but this can be overruled by the Environmental Assessment Office (Environmental Assessment Act, 2002). Often,

rocky intertidal habitats are overlooked by environmental campaigns and conservation organizations because this ecosystem is generally thought to be robust and resilient (Thompson et al., 2002). The intertidal zone, lying at the interface of land and sea, thus presents an interesting challenge for environmental protection, as the potential sources of pollution and damage are two-fold, originating from terrestrial or marine environments. Many policy and regulation reviews assess ecosystems broadly with limited or no consideration of the different requirements of species (Nowlan, 1999). The impacts of climate change on rocky intertidal ecosystems will be even more challenging for policy makers, as these impacts are predicted to vary geographically (Iwabuchi unpublished, Thorner et al. 2014). This means it will be difficult for regulation to effectively cover all types of shoreline with a single overarching legislation. Additionally, to my knowledge, no provincial regulations address the impacts of elevated temperature or desiccation on any intertidal communities, despite evidence that modest changes in climate might be sufficient to affect these populations. Based on the findings of this study, it's clear that marine animals are not only susceptible to changes in ocean temperature and chemistry but also to abiotic changes to aerial conditions. Thus, there is a clear need for protections of intertidal communities to incorporate conditions that these animals face on the interface of both land and sea. Rather, policy focuses on the loss of habitat from erosion and sea level rise, coastal development, ocean acidification, seawater temperature and the subsequent invasion of equatorial species poleward.

The discovery by the present study of significant interspecific variation in temperature and desiccation tolerance among intertidal invertebrates reveals a need to focus on the potentially divergent effects of increasing air temperature on populations of these species. In addition, these findings indicate that research of climate change impacts on coastal areas must be carried out at the species level rather than examine entire ecosystems, as the differences in tolerance to climate-induced stress among species can ultimately result in much broader changes in the ecosystem. For example, a rise in maximum air temperatures by only a few degrees might be lethal for a vulnerable species but not for a more tolerant species. Vulnerable species, however, often play important roles in community dynamics, be it through competition for space and resources or as a prey item for other species. As identified by this study, *M. trossulus* is a species that is particularly vulnerable to temperature

and desiccation stress in their early benthic phase. If temperatures were to increase so that *M. trossulus* populations could not persist, the reduction or disappearance of this species could alter the physical structure of rocky intertidal habitats; this could limit the availability of cryptic microhabitats for species such as *N. ostrina* and *P. cinctipes*, while increasing the availability of open space for barnacles. In turn, those changes could limit algal food availability for grazing species such as the littorine snail, *L. scutulata*, and limpets. Therefore, a small rise in temperature may not affect all species in the ecosystem through direct mortality, but if conditions are lethal for even one species highlighting the importance of framing policy around the “weakest link” or the species least tolerant to the stressors in question.

Air temperatures in British Columbia have increased 1.4°C in the last century; greater than that of the global average (Lemmen, 2016). With continued emissions of CO₂ worldwide, it is anticipated that the stress caused by high air temperatures and desiccation will increase. Thus there is a need for protection of rocky intertidal communities, not only from acidification, pollution and sea level rise, but also from rising air temperatures.

This study also revealed that, in all species tested, juveniles were always more vulnerable to elevated temperature and desiccation than adults. Therefore, any attempts to conserve this ecosystem must take into account the lowest tolerance thresholds of the animals in question. Rising air temperatures as a result of climate change are immensely difficult to regulate, and regulations on CO₂ emissions are never directed at specific tolerance thresholds of animals. If regulations were set to address specific species, this study suggests that tolerance thresholds for juveniles are more important in setting useful regulations than adults. For example, since early benthic phase *M. trossulus* are the least tolerant of high temperature of any species tested, it would be best to set regulations using this group’s tolerance thresholds. As with the findings of this research, it may be best to set regulations conservatively, using the least tolerant species to temperatures, *M. trossulus*, to inform policy. Since the early benthic phase has been revealed as a stage where animals are least tolerant to temperature and desiccation, regulations should aim to keep temperatures from rising beyond that which could be tolerant by juvenile *M. trossulus*. This is particularly important for species that reproduce during summer months because high air temperatures and desiccation could drastically alter population dynamics and community interactions

through high levels of early juvenile mortality. If there were an increase of hot days in coastal British Columbia, as projected by climate forecasts (Lemmen, 2016) there could be serious implications for rocky intertidal communities.

It may be unreasonable to expect policy makers to focus coastal regulations on particular life stages of individual species. To do so might be impractical and with the effects of rapidly intensifying climate change, timely policy must be implemented to ensure protection of rocky intertidal communities. This study however, also revealed that two lifestyle characteristics, microhabitat use and the upper extent of intertidal distribution, are correlated to the temperature and desiccation tolerance thresholds of intertidal invertebrates. Using these findings, we could hone research efforts to groupings of animals with shared characteristics shown to be related to stress tolerance. This would streamline the research process and allow for more informed generalisations of species rather than overarching policy, assuming the needs of all animals along the intertidal ecosystem are equal.

The publication “Canada’s Marine Coasts in a Changing Climate”, an assessment driven by Canada’s Adaptation Platform, addresses concerns for species living in mudflats, estuaries and beaches, but does not consider habitat loss or climate change impacts in rocky intertidal habitats (Lemmen, 2016). A need for stricter regulations and, at least some consideration to the impacts of climate change on intertidal zones is inarguable. The present study can inform policy makers by highlighting the need for a focus on interspecific and ontogenetic variation in tolerance to stress. Using tolerance limits determined by this study, British Columbia emission reduction targets could be set to limit temperature increases below the level that would pose lethal to the most vulnerable intertidal invertebrates. Many fluctuations in population abundance in the intertidal zone are the consequence of multiple stressors and trade-offs, thus shared characteristics would allow grouping of different species that may be more or less susceptible to certain types of stress. Focussing on intertidal distributions and microhabitat use of species could remove the need for challenging studies of the early benthic phase and extensive multi-species studies if the general ecology of the species in an ecosystem is known.

Directions for Future Study

To implement productive regulations protecting BC's coastlines, researchers must first develop a solid understanding of the natural variation in environmental conditions occurring in rocky shore ecosystems. Without a comprehensive understanding of baseline population dynamics, it will be exceedingly challenging to determine whether population fluctuations are caused by external or human-made conditions. Given the significant interspecific variation in temperature and desiccation tolerance revealed by this study, future research should investigate whether the species that were most tolerant of high temperature and desiccation are also the most tolerant to other stressors, such as low pH, water temperature and pollutants. This kind of study would provide further insight to the lifestyle characteristics that may be correlated to physiological tolerance to other stressors. Identifying these similarities among groups of animals could streamline policy planning and reduce the need of single species studies.

From previous studies, and now the findings of ontogenetic variation in physiological stress tolerance demonstrated in this study, it is clear that the early benthic phase for many marine invertebrates is a critical stage. This suggests that certain periods in an animal's life, and thus in the life cycle of a species, are more important in dictating population fluctuations than others. Knowing precisely when these changes occur in an animal's life, could better inform policy makers for these intertidal communities with the knowledge of periods in life and throughout the year which are most critical for a species. Since this study has determined that microhabitat use and intertidal distribution are related to temperature and desiccation tolerance, further research should investigate the timing of ontogenetic shifts in tolerance and in these lifestyle characteristics. Better understanding the relationship between these characteristics and an animal's tolerance will help ecologists and policy makers identify species most vulnerable to high stress and changes in environmental conditions associated with climate change.

LITERATURE CITED

Environmental Assessment Act [SBC 2002] Chapter 43

- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar Ecol Prog Ser* 155:269–301
- Lemmen DS, Warren FJ, James TS, Mercer Clarke, C.S.L editors (2016): *Canada's Marine Coasts in a Changing Climate*; Government of Canada, Ottawa ON
- Nowlan, L (1999) *Preserving British Columbia's Coast - A Regulatory Review*; East Coast Environmental Law for the BC Near Shore Habitat Loss Work Group, Victoria, BC
- Thompson R, Crowe T, Hawkins S (2002) Rocky intertidal communities: Past environmental changes, present status and predictions for the next 25 years. *Environ Conserv* 29:168–191
- Thorner J, Kumar L, Smith SDA (2014) Impacts of climate-change-driven sea level rise on intertidal rocky reef habitats will be variable and site specific. *PLoS One* 9:1–7

Appendix A. Size regression analysis of six intertidal invertebrates.

The relationship between linear measurements and body mass was determined by collecting 40 individuals of each species from Dixon Island in September 2016. Animals were returned to the laboratory where they were placed in water for 2 h to ensure full hydration of body tissues. Animals were removed from water, blot dried and weighed using an A&D ER-120A analytical balance measuring to the nearest 0.0001 g. Animals were dried at 60°C in a BOD® Low Temperature Incubator #2015 until a stable mass was reached. Stable mass was determined by repeatedly weighing organisms until a change in body mass less than 2% was observed between consecutive weighing. Body length was measured using a General Tools Dial Caliper measuring to the 0.254 mm. To minimize bias in the measurements, body length was measured three times and an average was used in calculations. Measurement error was calculated to be < 4.2% (Yezerinac et al., 1992) where a low percentage indicates a small proportion of variation in analysis is due to observer inconsistencies. All measurements were made blindly to previous measurements.

Linear regressions were generated from log transformed data, all resulting in strong correlation for both wet weights and dry weights. Wet weights has slightly stronger correlations than regressions using dry weights, therefore predicted weights for analysis of temperature and desiccation data were estimates of wet weight (Pearson's correlation > 0.96).

Forty individuals of each species were measured at a defined morphological markers for body length and data was log-transformed. The following table provided regression equations and associated statistic of fit.

Table A.1. Least squares regressions for correlation between log-transformed body length and wet weight of six intertidal invertebrates.

Species	N	Equation	R ²	Residual Std. Err.
<i>M. trossulus</i>	40	$y = 2.85x - 3.72$	0.980	0.076
<i>N. ostrina</i>	40	$y = 2.98x - 3.84$	0.993	0.062
<i>C. dalli</i>	40	$y = 2.94x - 3.72$	0.933	0.110
<i>B. glandula</i>	40	$y = 3.53x - 3.97$	0.928	0.120
<i>L. scutulata</i>	40	$y = 2.86x - 3.52$	0.958	0.063
<i>P. cinctipes</i>	40	$y = 3.22x - 3.14$	0.990	0.049

Body length measurement were repeated three times to ensure variance was not a result of measurement error. The percentage of variation due to measurement discrepancies was calculated using the equation

$$\%ME = \frac{s_{within}^2}{s_{within}^2 + s_{among}^2} \times 100,$$

adapted from (Yezerinac et al., 1992). Percent measurement error was low for all species (Table 2) and variance in measurements did not show any trends correlated to size.

Table A.2. Percentage of variance due to variation in measurement as calculated by Yezerinac et al. (1992) (n=3).

Species	%ME
<i>M. trossulus</i>	0.060
<i>N. ostrina</i>	0.289
<i>C. dalli</i>	4.193
<i>B. glandula</i>	3.335
<i>L. scutulata</i>	3.078
<i>P. cinctipes</i>	1.123

Where residual standard error of regressions is low, the low percentage of variance due to measurement error is acceptable and suggests measurements were precise and consistent with all species.

Appendix B. Sigmoidal regressions of survival after temperature or desiccation treatments

Following exposure to temperature or desiccation treatments, animals were monitored for survival and a sigmoidal regression was fit to these data for each life stage of each species. Using a generalized linear model with a binomial distribution the model allowed for observations that don't fit a binomial distribution. In some cases, Bayesian statistical methods had to be used to account for perfect separation of the data (Gelman et al., 2008). Only examples of these regressions were provided in the results section of the thesis. Following are all regression for temperature and desiccation data of each life stage of each species tested. Curved lines represent the logistic regression fit to survival data and the horizontal line intersects with the curve where %50 of individuals die.

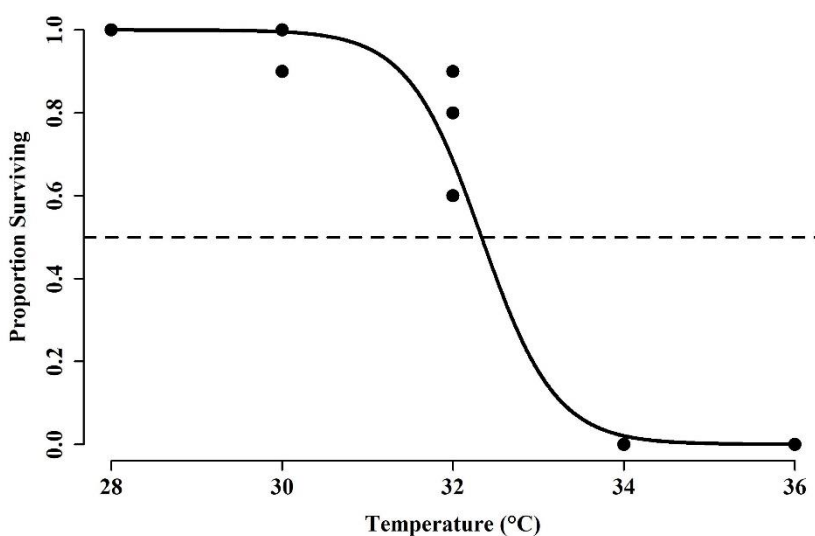


Figure B.1. Effect of 6 h temperature treatment on the survival of *N. ostrina* juveniles where each point represents the proportion of individuals surviving after a temperature treatment.

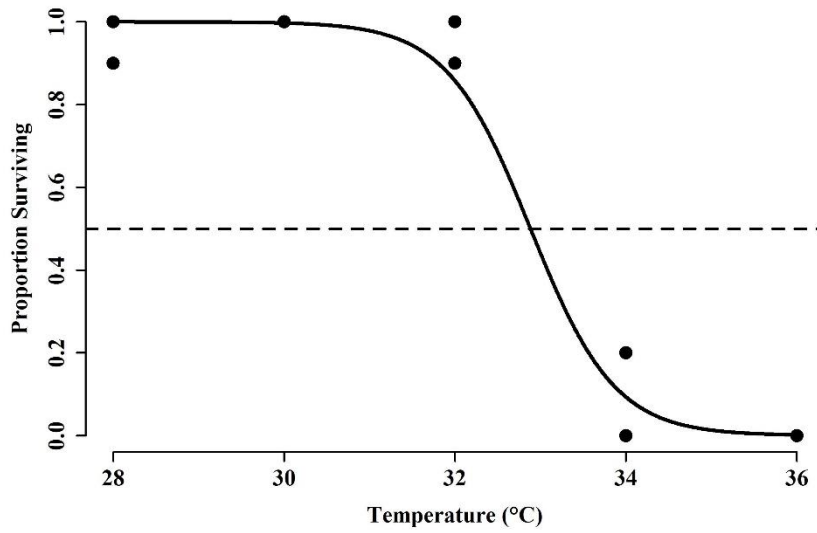


Figure B.2. Effect of 6 h temperature treatment on the survival of *N. ostrina* adults where each point represents the proportion of individuals surviving after a temperature treatment.

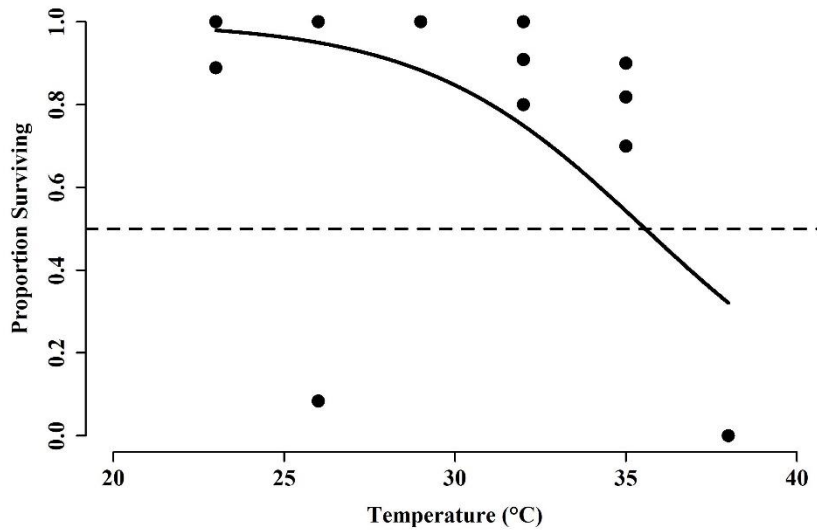


Figure B.3. Effect of 6 h temperature treatment on the survival of *M. trossulus* juveniles where each point represents the proportion of individuals surviving after a temperature treatment.

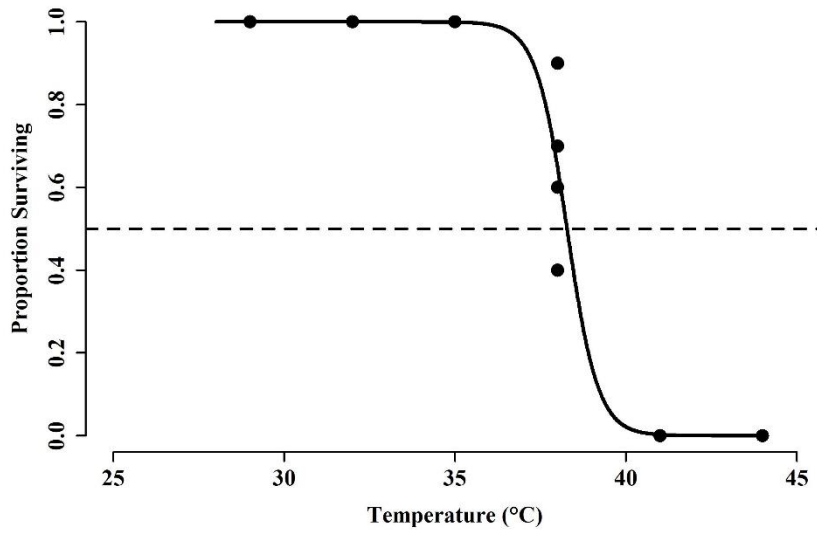


Figure B.4. Effect of 6 h temperature treatment on the survival of *M. trossulus* adults where each point represents the proportion of individuals surviving after a temperature treatment.

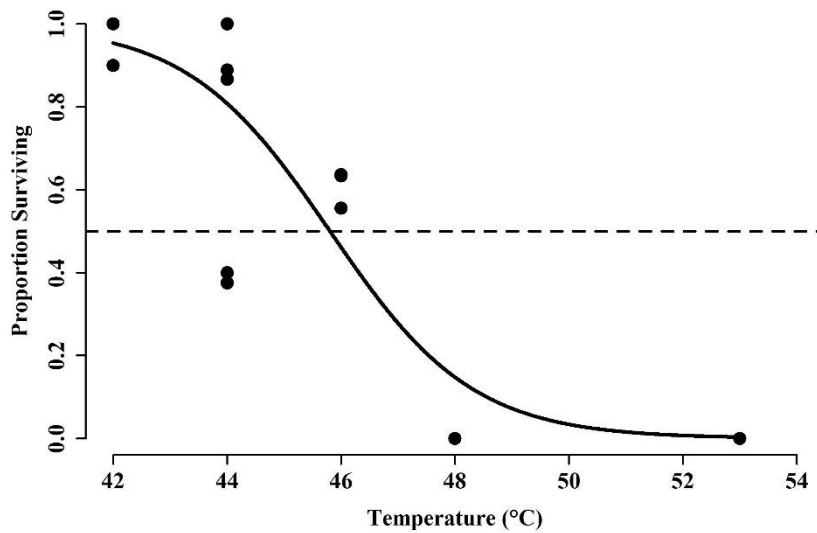


Figure B.5. Effect of 6 h temperature treatment on the survival of *C. dalli* juveniles where each point represents the proportion of individuals surviving after a temperature treatment.

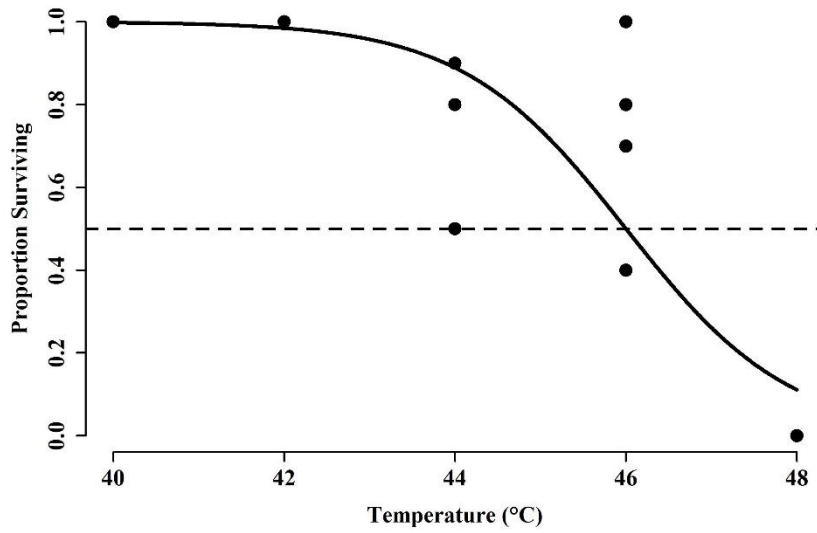


Figure B.6. Effect of 6 h temperature treatment on the survival of *C. dalli* intermediates where each point represents the proportion of individuals surviving after a temperature treatment.

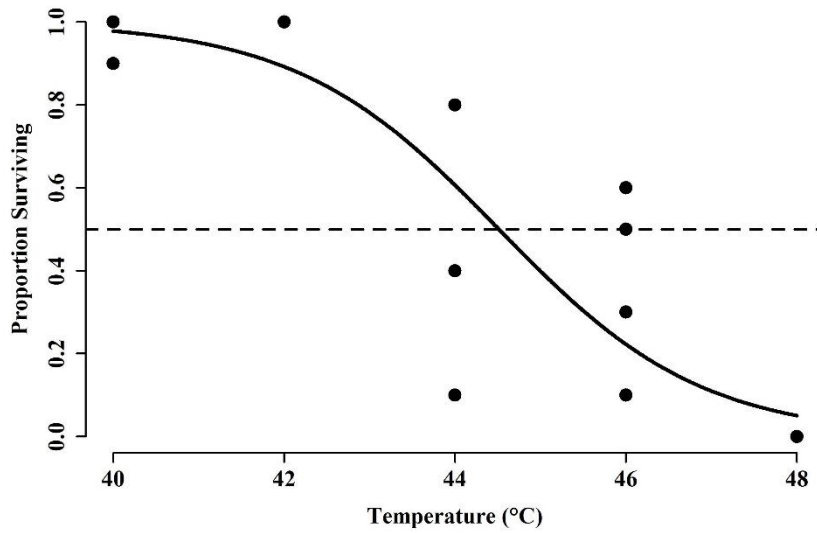


Figure B.7. Effect of 6 h temperature treatment on the survival of *C. dalli* adults where each point represents the proportion of individuals surviving after a temperature treatment.

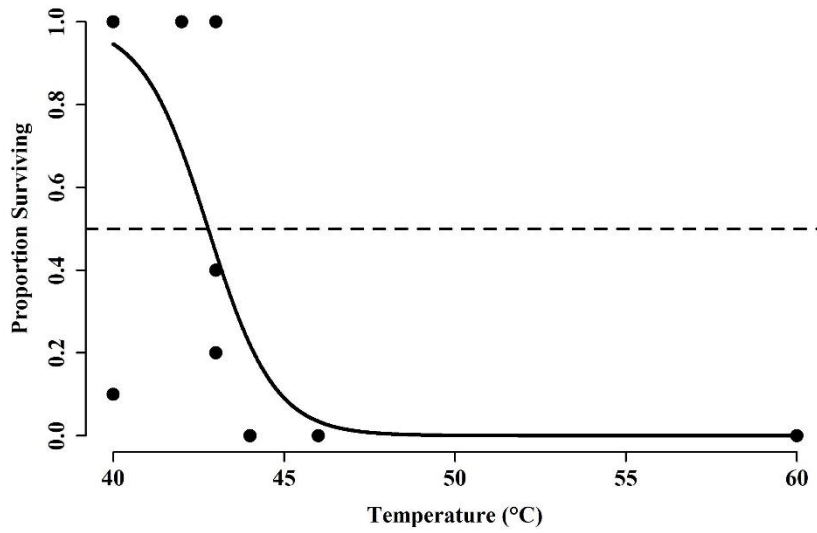


Figure B.8. Effect of 6 h temperature treatment on the survival of *B. glandula* adults where each point represents the proportion of individuals surviving after a temperature treatment.

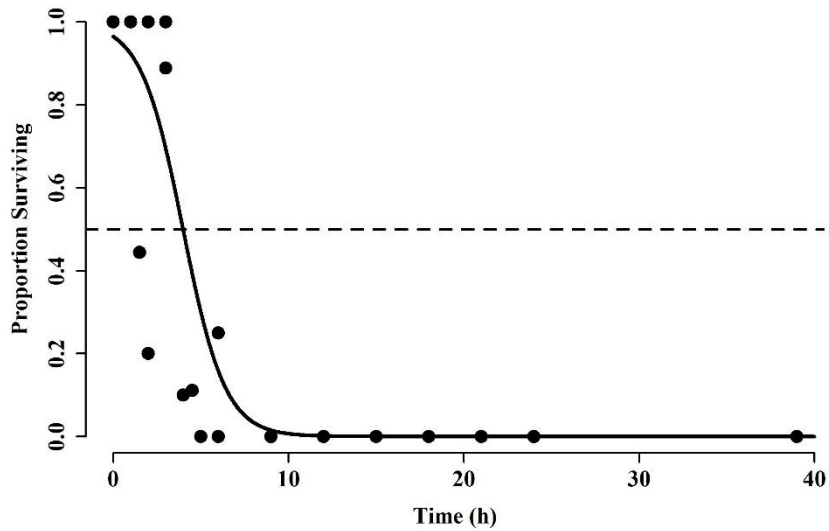


Figure B.9. Effect of desiccating conditions on the survival of *N. ostrina* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

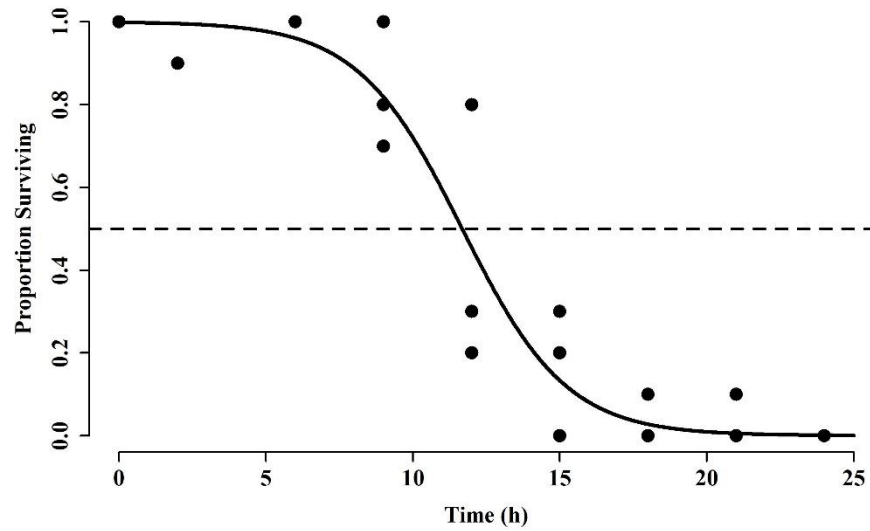


Figure B.10. Effect of desiccating conditions on the survival of *N. ostrina* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

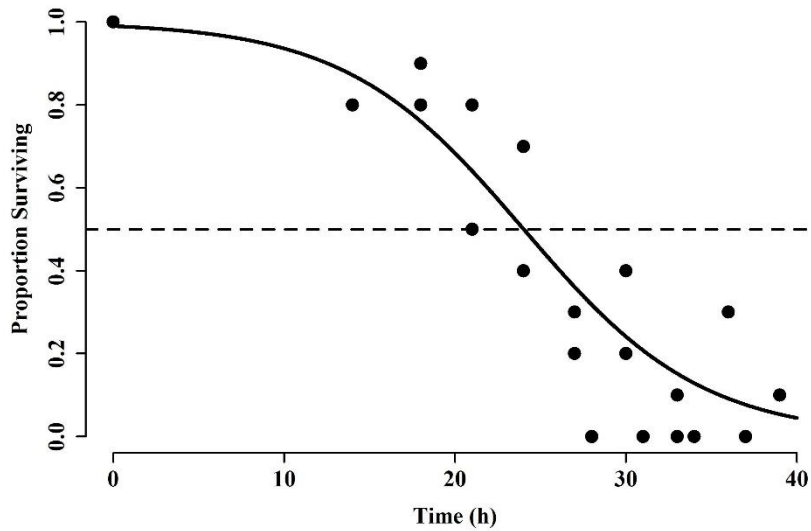


Figure B.11. Effect of desiccating conditions on the survival of *N. ostrina* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

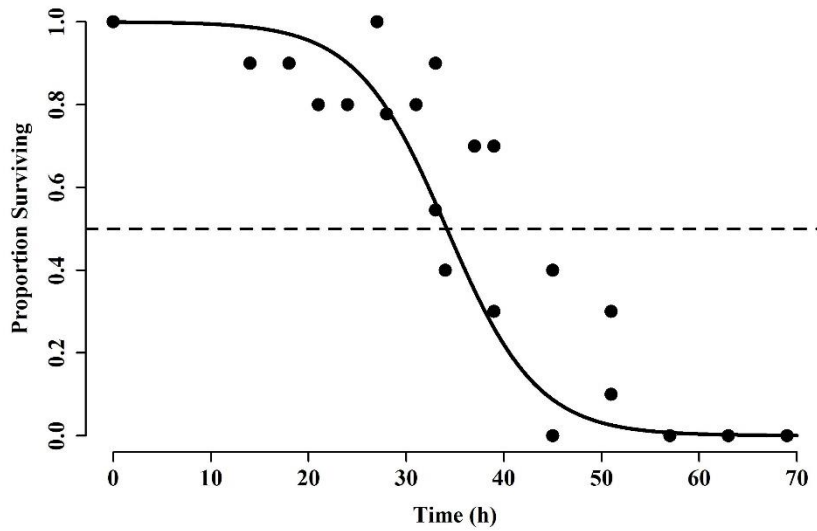


Figure B.12. Effect of desiccating conditions on the survival of *N. ostrina* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

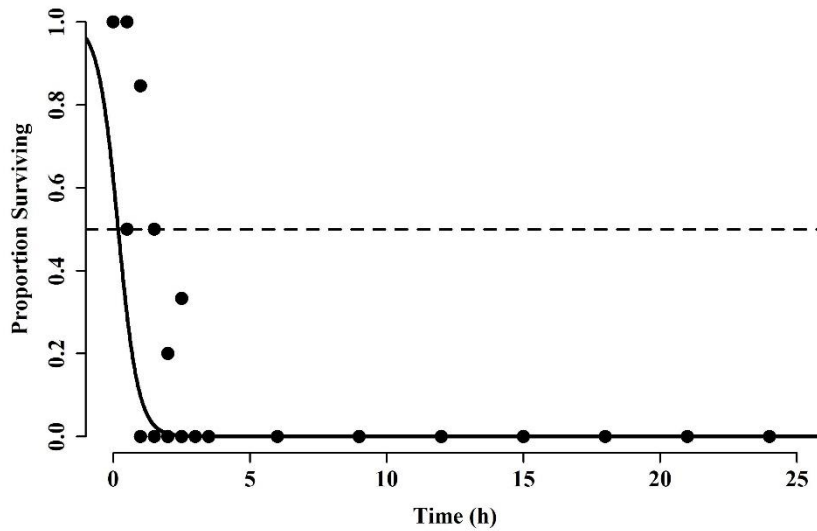


Figure B.13. Effect of desiccating conditions on the survival of *M. trossulus* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

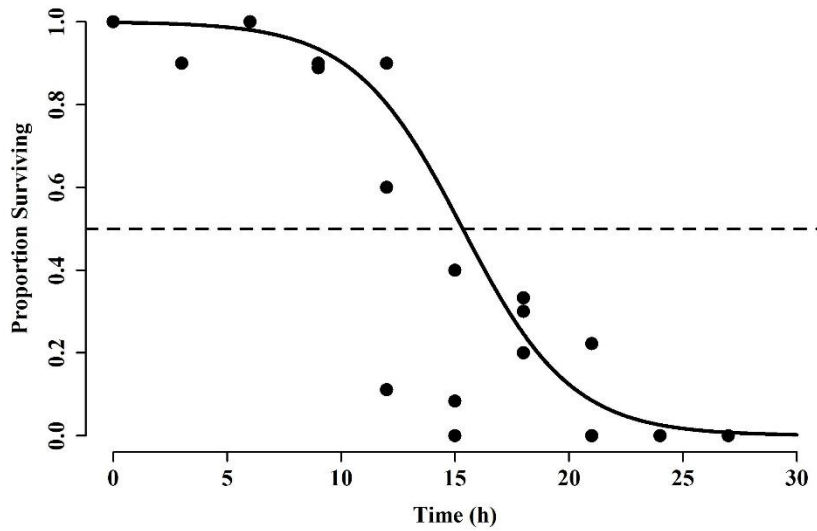


Figure B.14. Effect of desiccating conditions on the survival of *M. trossulus* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

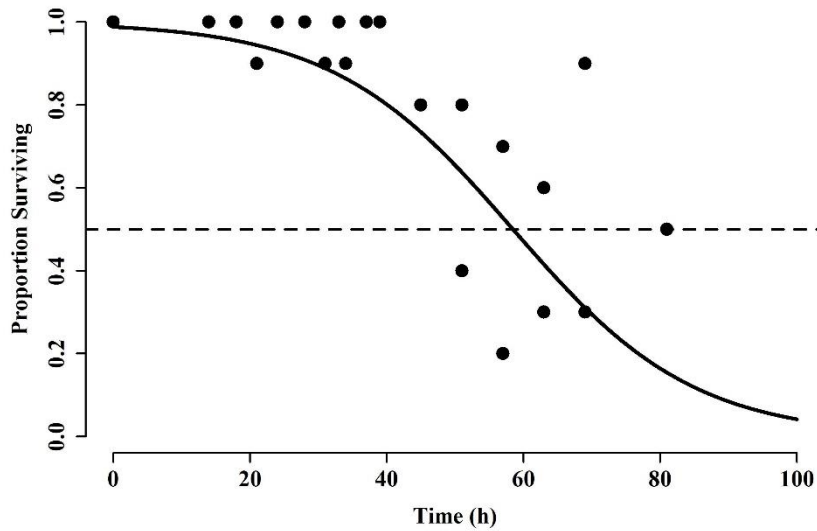


Figure B.15. Effect of desiccating conditions on the survival of *M. trossulus* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

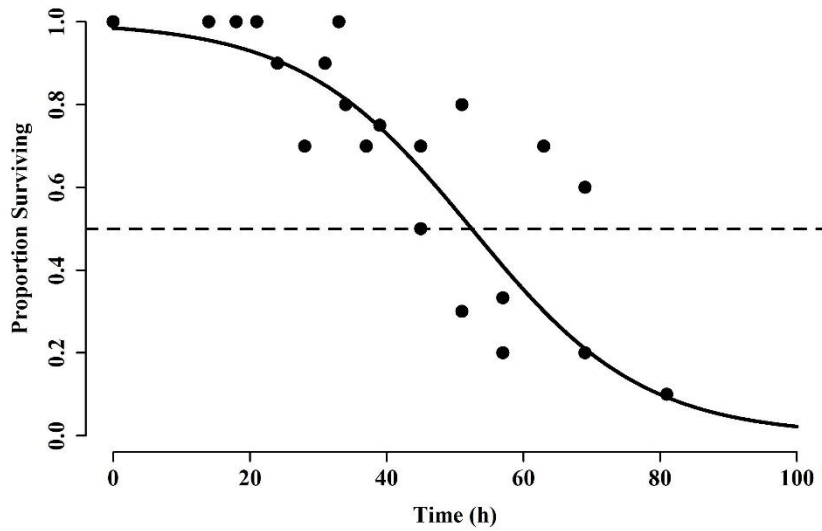


Figure B.16. Effect of desiccating conditions on the survival of *M. trossulus* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

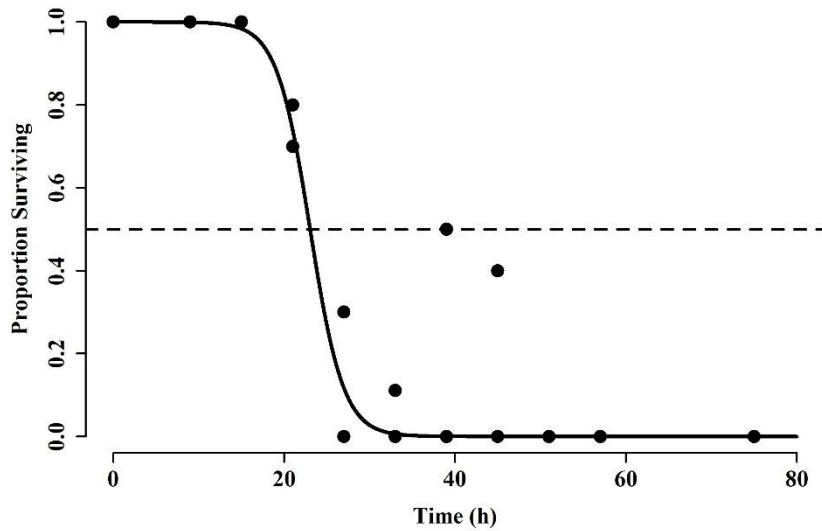


Figure B.17. Effect of desiccating conditions on the survival of *C. dalli* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

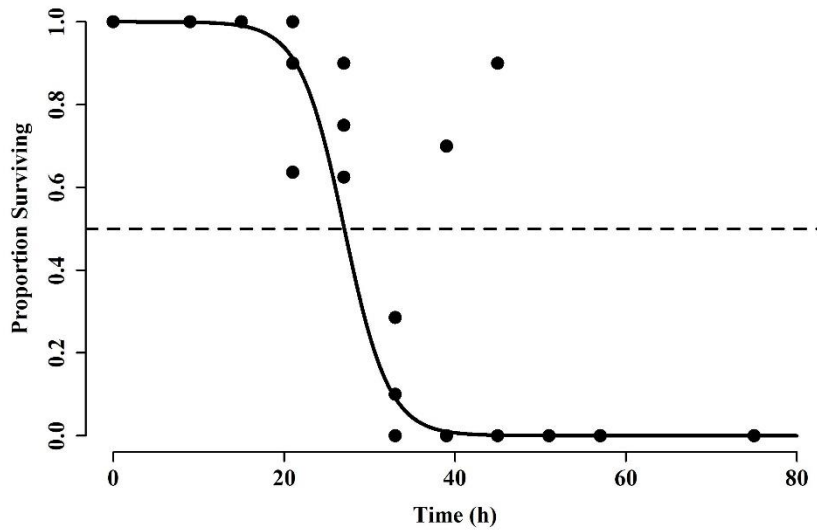


Figure B.18. Effect of desiccating conditions on the survival of *C. dalli* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

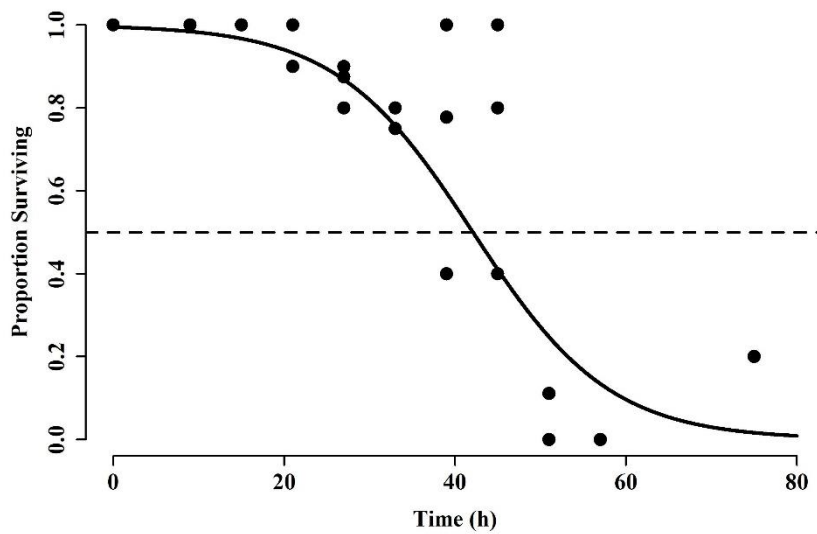


Figure B.19. Effect of desiccating conditions on the survival of *C. dalli* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

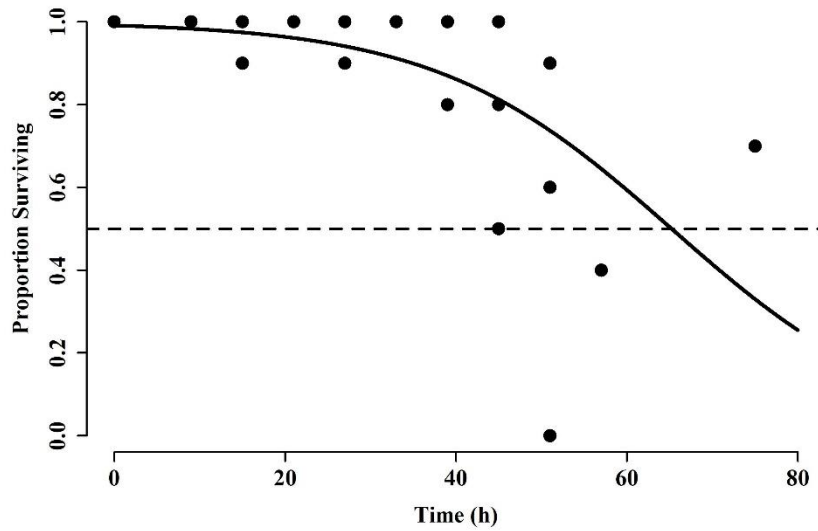


Figure B.20. Effect of desiccating conditions on the survival of *C. dalli* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

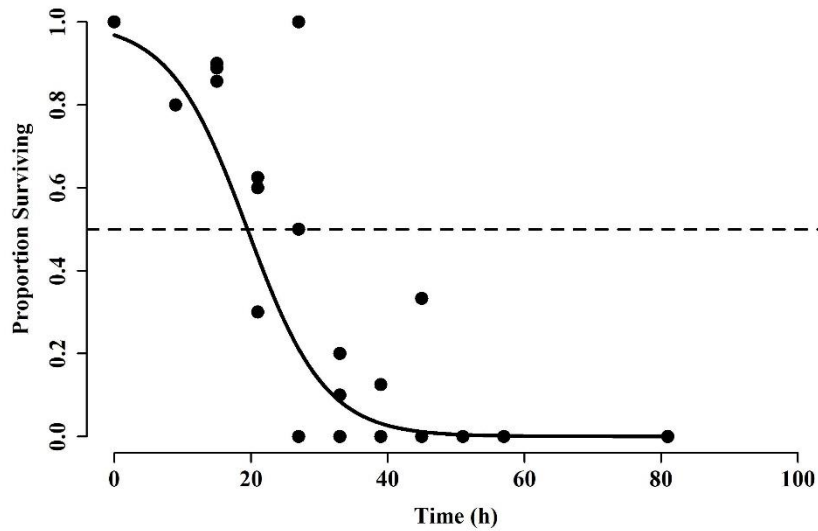


Figure B.21. Effect of desiccating conditions on the survival of *B. glandula* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

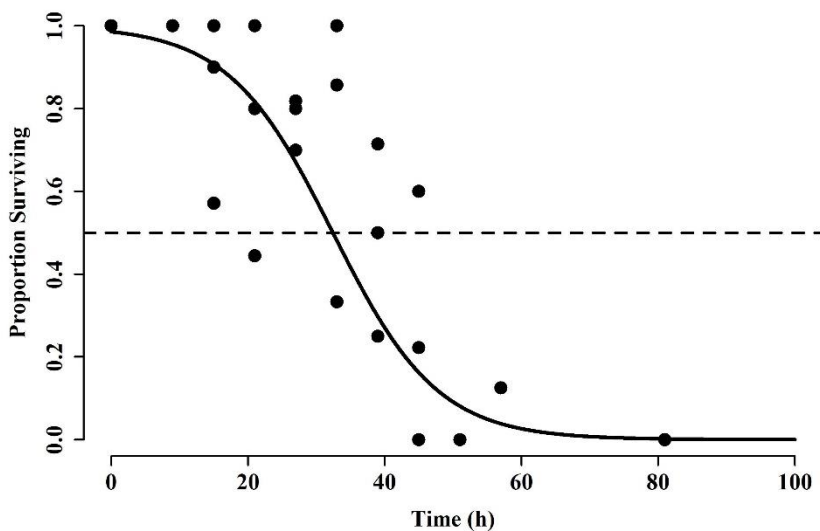


Figure B.22. Effect of desiccating conditions on the survival of *B. glandula* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

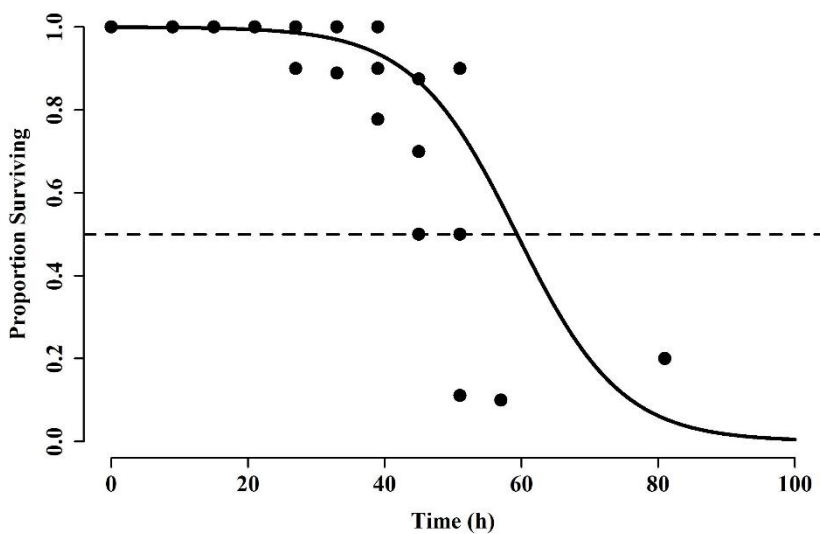


Figure B.23. Effect of desiccating conditions on the survival of *B. glandula* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

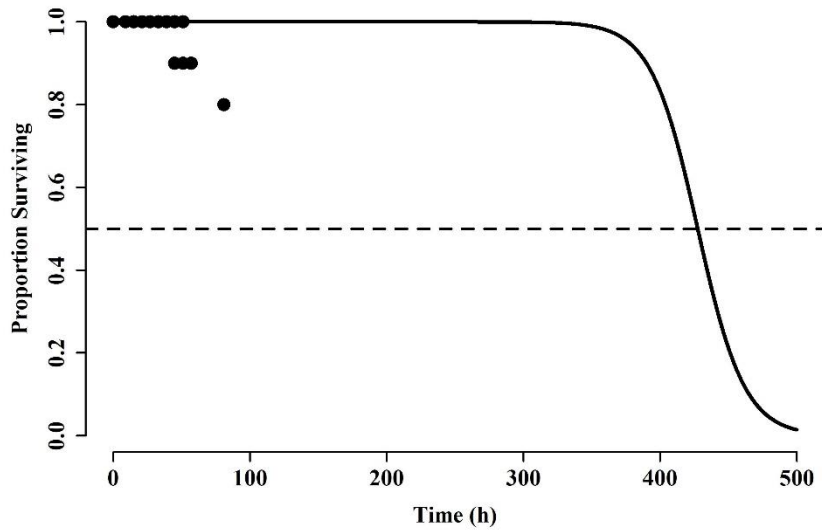


Figure B.24. Effect of desiccating conditions on the survival of *B. glandula* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

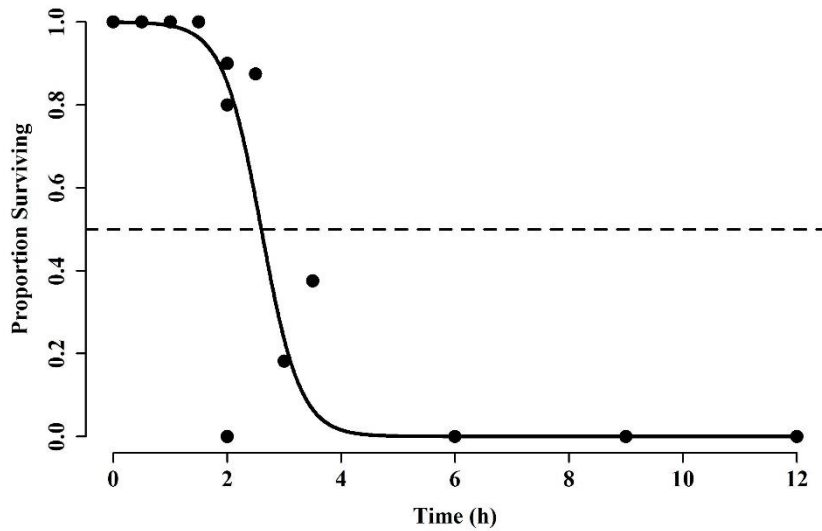


Figure B.25. Effect of desiccating conditions on the survival of *P. cinctipes* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

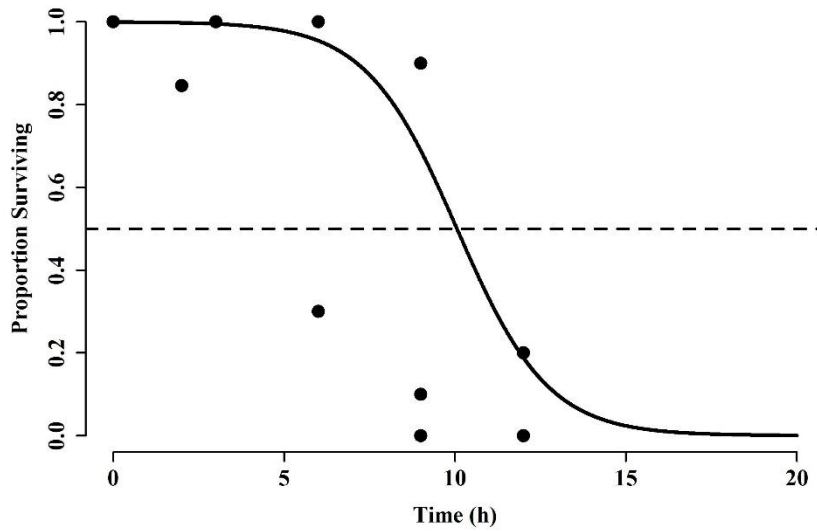


Figure B.26. Effect of desiccating conditions on the survival of *P. cinctipes* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

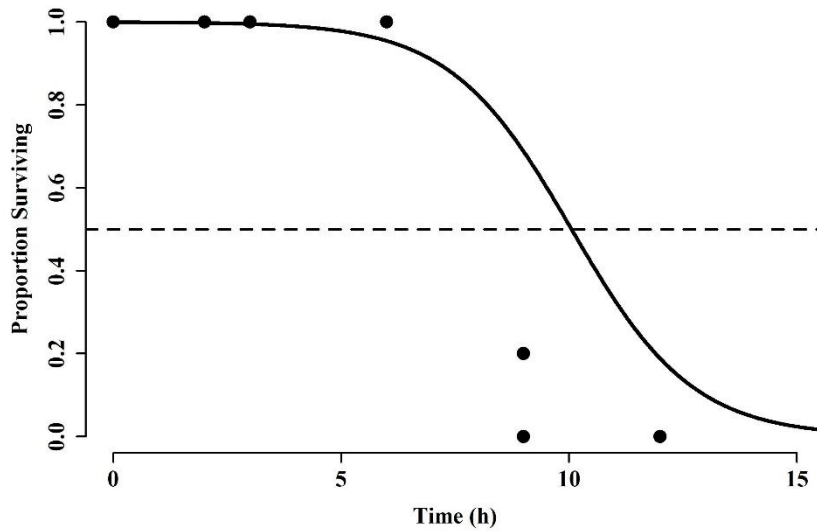


Figure B.27. Effect of desiccating conditions on the survival of *P. cinctipes* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

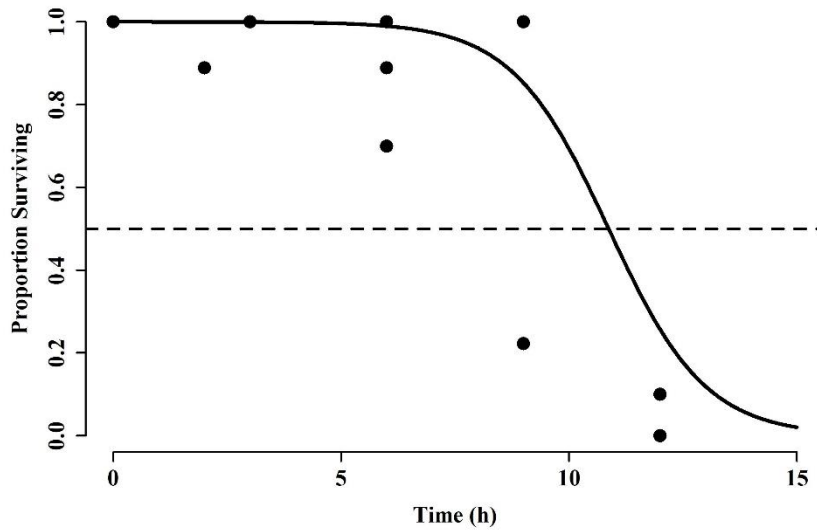


Figure B.28. Effect of desiccating conditions on the survival of *P. cinctipes* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

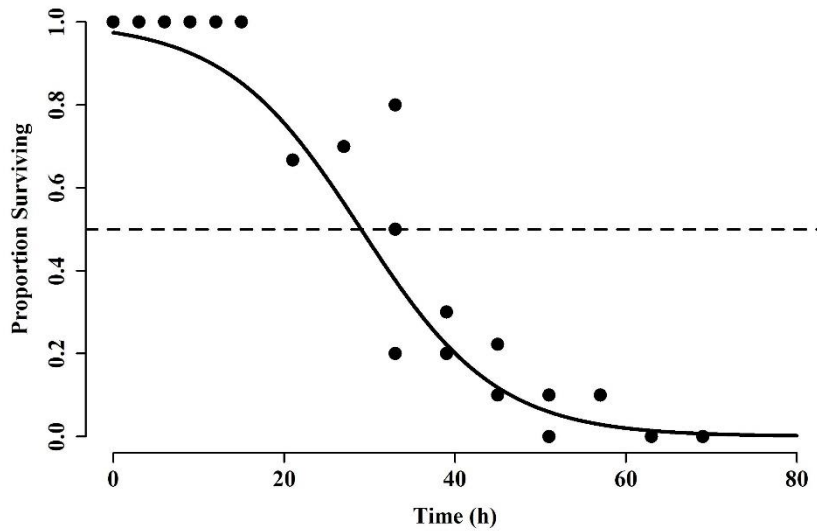


Figure B.29. Effect of desiccating conditions on the survival of *L. scutulata* size class A where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

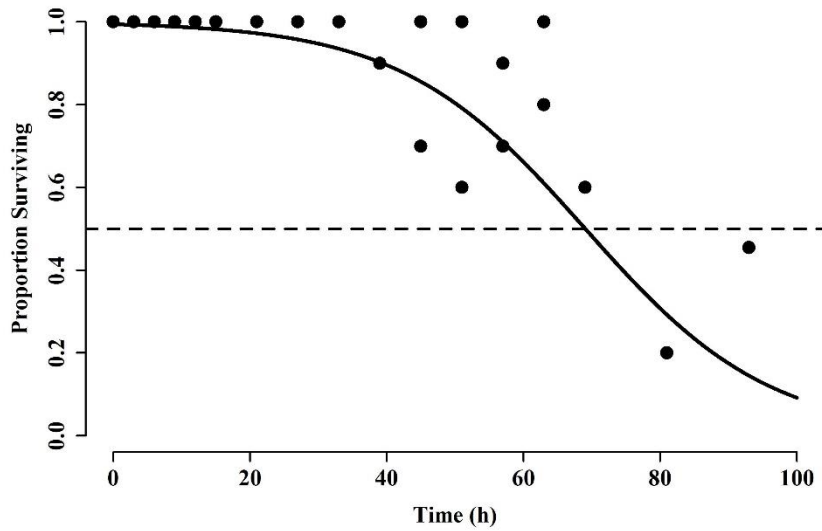


Figure B.30. Effect of desiccating conditions on the survival of *L. scutulata* size class B where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

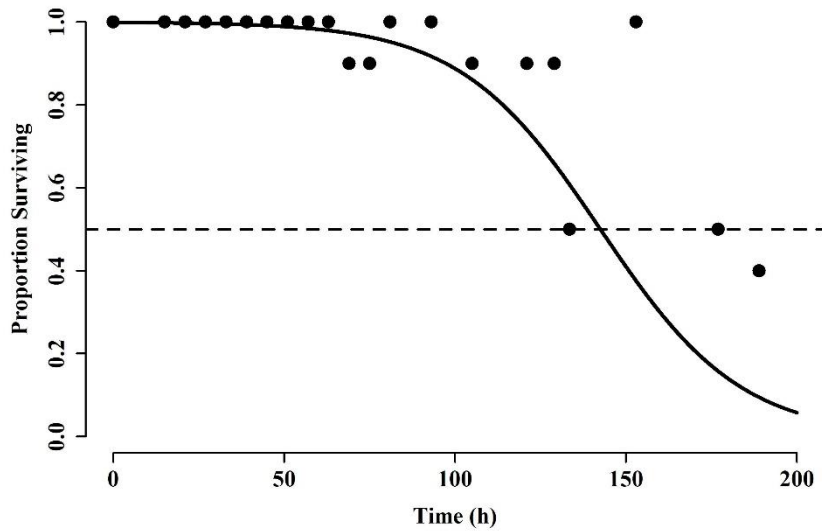


Figure B.31. Effect of desiccating conditions on the survival of *L. scutulata* size class C where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

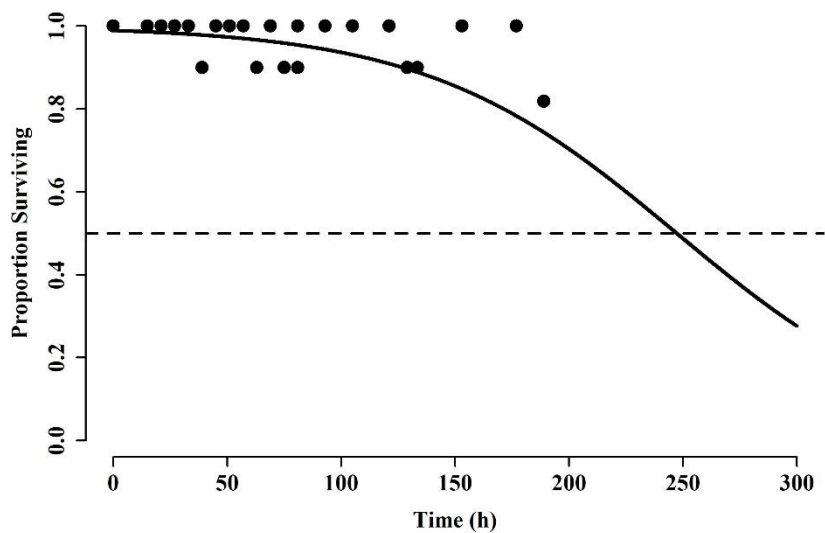


Figure B.32. Effect of desiccating conditions on the survival of *L. scutulata* size class D where each point represents the proportion of group of individuals surviving after a specific duration (h) at desiccation treatment.

Appendix C. Geometric relationship between volume and surface area to volume ratio.

To the relationship between an animal's volume and their surface area to volume ratio, several basic geometric shapes were used to characterize the shape of the relationship. For all shapes, sphere, cone and cube, as the volume as an animal increases, the surface area to volume decreases. In all cases, surface area to volume ratio decreases rapidly with volume and the rate of decrease slows with larger volume.

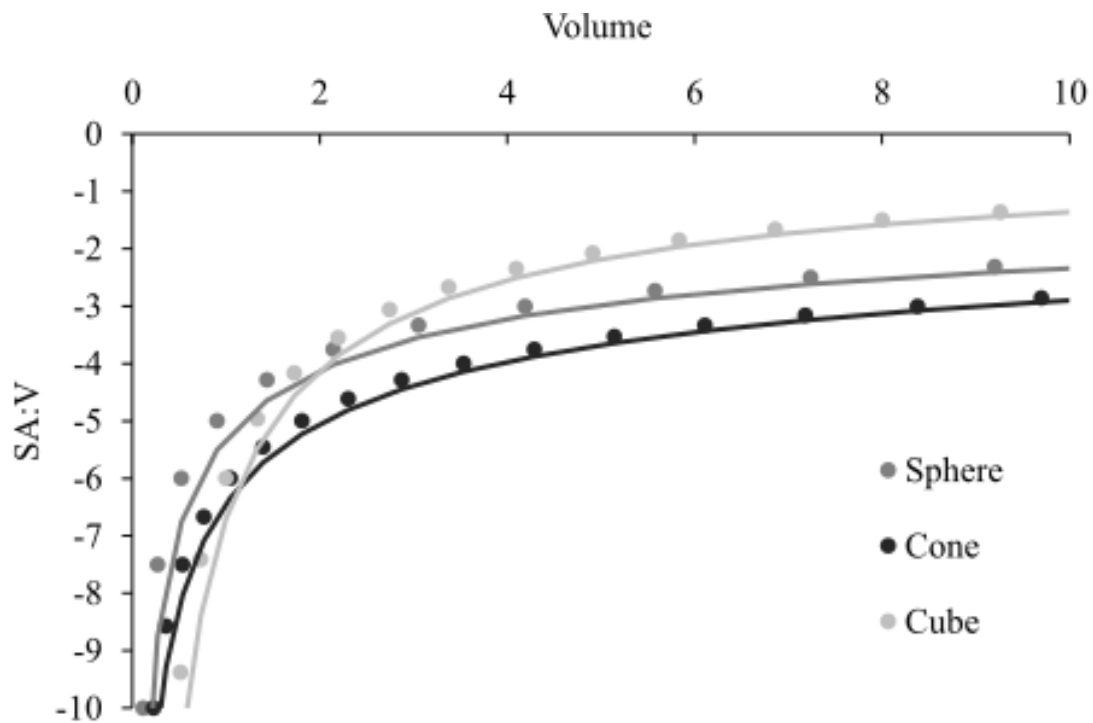


Figure C.1. Graphic illustration of the relationship between surface area to volume ratio and the volume of several three dimensional shapes.

Appendix D. Correlation analysis between lifestyle characteristics and tolerance thresholds.**Table D.1.** Summary of correlations between lifestyle characteristics and tolerance thresholds from Pearson's product-moment correlation analysis using log-transformed data. * indicates data that was analyzed with untransformed data.

Stress	Life Stage	Factors	R²	n	p
Temperature	Juvenile	LT ₅₀ vs Intertidal Height	0.86	4	0.07
		LT ₅₀ vs Microhabitat	0.85	4	0.078
		LT ₅₀ vs Motility	0.85	4	0.078
		Intertidal height vs Microhabitat	0.95	4	0.023
		Intertidal height vs Motility	0.95	4	0.023
		Microhabitat vs Motility	1	4	< 0.001
	Adult	LT ₅₀ vs Intertidal Height	0.87	4	0.068
		LT ₅₀ vs Microhabitat	0.73	4	0.14
		LT ₅₀ vs Motility	0.73	4	0.14
		Intertidal height vs Microhabitat	0.53	4	0.27
		Intertidal height vs Motility	0.51	4	0.28
		Microhabitat vs Motility	1	4	< 0.001
Desiccation	Juvenile	* LT ₅₀ vs Intertidal Height	0.63	6	0.0595
		LT ₅₀ vs Microhabitat	0.72	6	0.03
		LT ₅₀ vs Motility	0.08	6	0.58
		Intertidal height vs Microhabitat	0.67	6	0.045
		Intertidal height vs Motility	0.62	6	0.06
		Microhabitat vs Motility	0.34	6	0.22
	Adult	LT ₅₀ vs Intertidal Height	0.77	6	0.02
		LT ₅₀ vs Microhabitat	0.62	6	0.06
		LT ₅₀ vs Motility	0.32	6	0.24
		Intertidal height vs Microhabitat	0.86	6	0.0062
		Intertidal height vs Motility	0.61	6	0.069
		Microhabitat vs Motility	0.72	6	0.031